Research Article

Composted Rice Husk Improves the Growth and Biochemical Parameters of Sunflower Plants

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The present study investigated the effects of composted rice husk (5 and 26; 10 g/2 kg of soil/pot) on growth and biochemical parameters of sunflower plants at the 30th and 60th day of germination. Result showed significant improvement in growth and biochemical parameters of plants as compared to control plants treated with uncomposted organic fertilizer. However, the effects vary with the microbial treatments involved in the composting of rice husk like composted with *T. hamatum* (JUF1), bradyrhizobium *sp-II* (JUR2) alone, and JUF1 in combination with *Rhizobium sp-I* (JUR1) were found effective in improving the shoot and root lengths, total chlorophyll, carbohydrate, crude protein, and mineral (nitrogen and phosphorus) content of sunflower plants. It indicates that composted rice husk with improved total carbohydrate and protein contents may increase the soil fertility by improving its organic content.

1. Introduction

Agriculture plays an important role in economy of developing countries like Pakistan. However, rapid crop production with inappropriate farming practices deteriorate organic matter in soil, which results in decreased microbial activity that eventually affect its physical, chemical, and biological conditions [1] which leads to decline in land productivity and crop yields. To solve this problem, synthetic fertilizers were always thought to be a better way to improve the soil fertility and crop productivity but unfortunately the excessive use of these creates a number of serious environmental and health risks [2]. To minimize these hazards, naturally occurring organic fertilizers, namely, animal and plant manures, fall residues, and food and urban wastes are better alternate of commercially available fertilizers. Reports proved that organic farming improves soil composition, fertility, and soil fauna which in the long run have a beneficial effect on crop production [3]. For example, organic modification of soil with rice husk was found effective in the yield of many crops like cowpea and rice which saved 31–70% *Parkia biglobosa* from wilting caused by *Fusarium solani* and under diverse

irrigation period can give good rice stand, improved grain yield, and higher water use efficiency [4, 5].

Nowadays, composting is a system of recycling the organic waste matters in extradiigestible form with improved nutrient and mineral content by using beneficial microorganisms under specified conditions of temperature and aeration that could be used as compost or organic fertilizers which helps to recover the soil fertility and upgrade the crop growth [6]. Therefore, the present work was designed to investigate the effect of composted rice husk on physical and biochemical parameters of sunflower (*Helianthus annuus* L.; family Compositae), one of the most important oilseed plants [7]. Beside this, it is also involved in phytoremediation and used to extract toxic ingredients such as lead, arsenic, and uranium from soil [8].

2. Material and Methods

2.1. Microorganisms. Microorganisms including *Trichoderma hamatum* (JUF1) and *Rhizobium* species, namely, *Rhizobium sp. I* (JUR1) and *Bradyrhizobium sp. II* (JUR2) were

TABLE 1: Treatments of test microorganism alone and in combination used to prepare composted organic fertilizer.

S. no.	Treatments	Code
1	Control	Control
2	<i>Rhizobium sp</i> -I (10^{11} – 10^{12} cfu per mL)	JUR1
3	<i>Bradyrhizobium sp</i> -II (10^{11} – 10^{12} cfu per mL)	JUR2
4	<i>Trichoderma hamatum</i> (10^{11} – 10^{12} cfu per mL)	JUF1
5	<i>Rhizobium sp</i> -I (10^{11} – 10^{12} cfu per mL) + <i>T. hamatum</i> (10^{11} – 10^{12} cfu per mL)	JUR1 + JUF1
6	<i>Bradyrhizobium sp</i> -II (10^{11} – 10^{12} cfu per mL) + <i>T. hamatum</i> (10^{11} – 10^{12} cfu per mL)	JUR2 + JUF1

TABLE 2: Total carbohydrate and protein contents of composted rice husk after 15 days incubation.

S. no.	Treatments	Composted rice husk	
		Total protein ($\mu\text{g/g}$)	Total carbohydrate ($\mu\text{g/g}$)
1	Control (uncomposted)	10.59 ± 1.63	524.98 ± 12.04
2	JUR1	18.45 ± 2.60^d	598.97 ± 15.05^c
3	JUR2	14.74 ± 2.95	558.03 ± 46.60
4	JUF1	16.93 ± 5.12	974.79 ± 43.87^a
5	JUR1 + JUF1	15.84 ± 2.85	550.69 ± 53.81
6	JUR2 + JUF1	20.91 ± 6.47^c	795.77 ± 47.96^a

Each value is a mean $\pm$ S.D (standard deviation) of 3 replicates. Means bearing superscripts in each column are significantly different with respective control at $P < 0.05$.

isolated by root plating and crushed nodule methods [9], identified, and stored in the cultural collection of the Department of Botany, Jinnah University for Women, Karachi Pakistan.

2.2. Seeds, Fertilizer, Fungicide, and Organic Waste. Seeds of sunflower were purchased from Old vegetable market, Hyderabad, Pakistan. Nitrogen phosphorus potassium (NPK) and carbendazim were used as fertilizer and fungicide in an amount of 2500 ppm each and purchased from dealer of Agrochemical, Old vegetative market, Karachi, Pakistan. Rice husk was purchased from local market, Saddar, Karachi, Pakistan and used to prepare composted organic fertilizer.

2.3. Procedure for Preparing Composted Organic Fertilizer. Rice husk (160 g) was taken in conical flask (500 mL) and inoculated with each of the test microorganism (10^{11} – 10^{12} cfu/mL) alone and in different combinations (Table 1) under sterilized condition. The concentration of each test microorganism was adjusted with the help of haemocytometer [10, 11]. Three replicates were made for each treatment and incubated for 15 days at room temperature. After incubation period, composted rice husk was oven dried at 80°C for 2 hours and grinded to use as composted organic fertilizer in pot experiment after estimating its total carbohydrate and protein contents by Anthrone [12] and Lowry's [13] methods, respectively.

2.4. Experimental Pot Design and Procedure. The randomized complete block designed pot experiment was conducted in net house of Department of Botany, Jinnah University for Women. Seeds of experimental plants were sown in pots filled with 2 kg soil in each. At the 7th day of germination of developing seedlings, composted rice husk of each treatment

in an amount of 5 and 10 g/pot was applied and irrigated with tap water. All the treatments were maintained for 60 days. The plants were harvested at the 30th and 60th day of their germination by uprooting one plant from each pot of each treatment. Finally the uprooted plants were subjected to physical, biochemical, and mineral content analysis. Five pots were used as replicates for each treatment along with five control (untreated) plants.

2.5. Physical and Biochemical Parameters. Physical parameters included root & shoot lengths and fresh plant weight (biomass) were recorded by using measuring scale and weighing balance respectively. The biochemical analysis was done by determining total chlorophyll and total carbohydrate content by standard methods [12, 14] while crude protein by multiplying percent nitrogen value through 6.25 [15]. The percent nitrogen and phosphorus were estimated by Nessler's method [16] and Barton reagent [17].

2.6. Statistical Analysis. Results are expressed as mean $\pm$ standard deviation (S.D.) and data was analyzed by using One-way ANOVA followed by LSD (least significant difference) test through SPSS 16. The differences were considered significant at $P < 0.05$ when treatments' means were compared with control.

3. Results

3.1. Effect of Treatments on Total Carbohydrate and Protein Contents of Composted Rice Husk after 15 Days Incubation. Only few treatments like JUR1, JUF1, and JUR2 + JUF1 enhanced the total carbohydrate and protein contents in composted rice husk as compared to uncomposted (control) rice husk after 15 days of incubation at $28^\circ\text{C} \pm 5$ (Table 2).

TABLE 3: Effect of composted rice husk on physical parameters of *H. annuus* (sunflower) plants.

		Root length (cm)		Shoot length (cm)		Fresh weight (gm)	
		30th day	60th day	30th day	60th day	30th day	60th day
5 g rice husk	Control	20.5 ± 0.50	23.96 ± 1.09	28.03 ± 1.35	31.76 ± 0.66	1.41 ± 0.21	1.46 ± 0.13
	JUR1	24.9 ± 1.47 ^d (+21.46)	29.66 ± 5.00 (+23.78)	34.86 ± 1.91 ^a (+24.36)	47.36 ± 3.68 ^a (+49.11)	2.27 ± 0.29 (+60.99)	3.27 ± 1.15 ^d (+123.97)
	JUR2	25.5 ± 2.76 ^d (+24.39)	28.6 ± 1.55 (+19.36)	34.03 ± 3.18 ^b (+21.4)	44.13 ± 3.09 ^a (+38.94)	3.11 ± 0.81 ^b (+120.56)	3.37 ± 0.56 ^c (+130.82)
	JUF1	28.0 ± 2.29 ^b (+36.58)	28.63 ± 0.77 (+19.49)	36.96 ± 1.86 ^a (+31.85)	46.6 ± 1.70 ^a (+46.72)	3.24 ± 0.65 ^a (+129.78)	3.66 ± 0.57 ^c (+150.68)
	JUR1 + JUF1	25.43 ± 2.47 ^d (+24.04)	25.7 ± 5.00 (+7.26)	25.43 ± 2.47 (-9.27)	44.56 ± 2.79 ^a (+40.3)	2.13 ± 0.78 (+51.06)	3.93 ± 0.37 ^b (+169.17)
	JUR2 + JUF1	24.23 ± 1.25 (+18.19)	27.13 ± 7.64 (+13.23)	35.93 ± 1.96 ^a (+28.18)	43.23 ± 0.20 ^a (+36.11)	3.18 ± 0.40 ^a (+125.53)	3.37 ± 0.44 ^c (+130.82)
10 g rice husk	Control	25.03 ± 0.70	26.73 ± 2.95	25.33 ± 0.58	32.06 ± 2.10	1.57 ± 0.28	1.38 ± 0.44
	JUR1	28.1 ± 7.36 (+12.26)	30.16 ± 2.84 (+12.83)	40.86 ± 6.21 ^a (+61.31)	39.70 ± 1.77 ^c (+28.83)	3.12 ± 2.32 (+98.72)	2.73 ± 0.50 (+97.82)
	JUR2	26.23 ± 3.62 (+4.79)	30.63 ± 0.40 (+14.59)	34.73 ± 0.64 ^a (+37.11)	35.0 ± 2.16 (+9.17)	1.93 ± 0.21 (+22.92)	2.05 ± 0.15 (+48.55)
	JUF1	27.4 ± 1.47 (+9.46)	32.60 ± 5.25 ^d (+21.96)	36.0 ± 0.26 ^a (+42.12)	44.43 ± 3.44 ^a (+38.58)	2.51 ± 0.19 (+59.87)	3.13 ± 0.81 (+126.81)
	JUR1 + JUF1	26.03 ± 3.69 (+3.99)	33.96 ± 1.65 ^d (+27.04)	43.66 ± 1.52 ^a (+72.36)	48.53 ± 5.8 ^a (+51.37)	3.29 ± 1.00 (+109.55)	6.19 ± 3.76 ^a (+348.55)
	JUR2 + JUF1	27.93 ± 2.20 (+11.58)	30.86 ± 4.57 (+15.54)	40.83 ± 3.30 ^a (+61.19)	46.2 ± 1.3 ^a (+44.1)	3.01 ± 0.25 (+91.71)	3.65 ± 0.45 ^d (+164.49)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $P < 0.05$ (LSD). Values within parenthesis represent percent increase or decrease (-) with respective control.

3.2. Physical Parameters of Plants. Rice husk (RH) composted with each of JUR1, JUR2, JUF1 and JUR1 + JUF1, (5 g/2 kg soil/pot) was found effective in increasing the root length of sunflower plants from 21 to 36% at 30th day as compared to control plants while RH composted with each of JUF1 and JUR1 + JUF1 (10 g) was found effective in increasing the root length of experimental plants from 22 to 27% (Table 3). On the contrary, RH composted with all treatments separately in both concentrations (5 and 10 g) induced prominent outcome on shoot length at both days except RH composted with JUR1 + JUF1 (5 g) on 30th day and composted with JUR2 (10 g) on 60th day was found active in increasing the same parameter of plants (Table 3).

RH composted with JUR2, JUF1, and JUR2 + JUF1 (5 g) improved the fresh weight of plants from 120 to 131%, 130 to 150%, and 125 to 131%, respectively on both 30th and 60th day as compared to control plants while the same amount of RH composted with JUR1 and JUR1+JUF1 was found effective in improving the fresh weight of test plants with 124 and 169%, respectively, on 60th day. However, RH composted with JUR1 + JUF1 and JUR2 + JUF1 (10 g) found active in improving the same parameter only on 60th day (Table 3).

3.3. Photosynthetic Pigment of Leaves. RH composted with JUR2 (5 g) produced considerable improvement in total chlorophyll content from 36 to 41% in leaves of test plants on both 30th and 60th days. Whereas RH composted with JUF1

(5 and 10 g) was found efficient in improving the photosynthetic pigment from 40 to 88% on 60th day. RH composted with JUR1 + JUF1 (10 g) was found helpful in boosting the same parameter from 76 to 102% on both days (Table 4).

3.4. Biochemical Parameters. RH composted with all treatments (5 g) separately produced significant increase in carbohydrate content from 46 to 243% in sunflower plants on 30th day. In the same way, RH composted with JUF1 and JUR2 (10 g) was also found effective on 30th and 60th day in the same aspect (Table 4). RH composted with JUF1 (5 g) was found more efficient on 30th day in improving the crude protein content with 239% in plants than 10 g of same composted organic material that increased only 43% crude protein on the same 30th day. RH composted with JUR2 (10 g) and with JUR1 + JUF1 (5 g) improved the crude protein content on 60th day (61%) and 30th day (173%), respectively (Table 4).

3.5. Mineral Content. RH composted with JUF1 in amounts of 5 and 10 g enhanced the nitrogen content as 236 and 40%, respectively, in plants on 30th day. Whereas RH composted with JUR2 (10 g) was found helpful in the same aspect on 60th day. RH composted with JUR1 + JUF1 has accelerated the nitrogen content with 170% in plant on 30th day (Table 5). RH composted with JUF1 in both amounts (5 and 10 g) increased the phosphorus content in experimental plants by 400% on

TABLE 4: Effect of composted rice husk on biochemical parameters of *H. annuus* (sunflower) plants.

		Total chlorophyll (mg)		Total carbohydrate (mg/g)		Crud protein (%)	
		30th day	60th day	30th day	60th day	30th day	60th day
5 g rice husk	Control	2.41 ± 0.06	2.19 ± 0.08	173.55 ± 25.28	184.02 ± 14.76	7.51 ± 0.76	7.96 ± 0.35
	JUR1	2.68 ± 0.30 (+11.2)	2.26 ± 0.12 (+3.19)	253.94 ± 37.42 ^c (+46.32)	204.17 ± 27.03 (+10.94)	11.73 ± 1.74 (+56.19)	9.42 ± 1.24 (+18.34)
	JUR2	3.40 ± 0.50 ^d (+41.07)	2.99 ± 0.14 ^d (+36.52)	261.87 ± 40.85 ^c (+50.89)	208.08 ± 13.67 (+13.07)	12.12 ± 1.89 (+61.38)	9.63 ± 0.62 (+20.97)
	JUF1	3.03 ± 0.81 (+25.72)	3.07 ± 0.30 ^c (+40.18)	595.71 ± 48.16 ^a (+243.25)	197.62 ± 17.46 (+7.39)	25.46 ± 5.22 ^a (+239.01)	9.15 ± 0.82 (+14.94)
	JUR1 + JUF1	3.03 ± 0.48 (+25.72)	2.38 ± 0.22 (+8.67)	443.06 ± 46.63 ^a (+155.29)	202.06 ± 4.92 (+9.89)	20.49 ± 6.78 ^a (+172.83)	9.34 ± 0.20 (+17.33)
	JUR2 + JUF1	2.60 ± 0.53 (+7.88)	2.68 ± 0.36 (+22.37)	270.75 ± 33.89 ^c (+56)	205.75 ± 3.85 (+11.8)	12.51 ± 1.57 (+66.57)	9.53 ± 0.16 (+19.72)
10 g rice husk	Control	1.75 ± 0.34	2.22 ± 0.49	218.86 ± 20.68	266.31 ± 22.86	9.71 ± 0.95	10.63 ± 1.92
	JUR1	1.77 ± 0.38 (+1.14)	2.35 ± 1.17 (+5.85)	248.3 ± 20.38 (+13.45)	277.62 ± 43.02 (+4.24)	11.64 ± 4.66 (+19.87)	13.52 ± 4.08 (+27.18)
	JUR2	1.22 ± 0.31 (+30.28)	3.13 ± 0.44 (+40.99)	243.8 ± 51.20 (+11.39)	369.35 ± 31.54 ^c (+38.69)	11.25 ± 2.35 (+15.85)	17.09 ± 5.97 ^d (+60.77)
	JUF1	2.06 ± 0.38 (+17.71)	4.17 ± 1.16 ^d (+87.63)	299.92 ± 46.02 ^c (+37.03)	253.58 ± 62.75 (-4.78)	13.87 ± 2.13 ^d (+42.84)	12.57 ± 5.24 (+18.25)
	JUR1 + JUF1	3.09 ± 0.51 ^d (+76.57)	4.48 ± 1.11 ^d (+101.8)	225.20 ± 47.55 (+2.89)	348.42 ± 58.96 ^d (+30.83)	10.42 ± 2.21 (+7.31)	16.1 ± 2.71 (+51.45)
	JUR2 + JUF1	2.19 ± 0.81 (+25.14)	3.86 ± 0.27 (+73.87)	179.44 ± 4.4 (-18.01)	275.51 ± 26.06 (+3.45)	8.29 ± 0.20 (-14.62)	12.73 ± 3.98 (+19.75)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $P < 0.05$ (LSD). Values within parenthesis represent percent increase or decrease (-) with respective control.

30th day and 89% on 60th day, respectively. Similarly, RH composted with JUR2 stimulated the phosphorus content of plants by 186% on 30th day (Table 5).

4. Discussion

Pakistan is one of the countries whose economy chiefly depends on agriculture, its more than 67% residents are living in rural areas and farming is their native occupation [18]. In order to fulfill the consumers demand, synthetic fertilizers are always the first preference of our farmers. However, these are reported to produce a number of injurious effects on the whole ecosystem including the environment, soil biota, and human and also influence the soil fertility [19]. To overcome these serious impacts, microbial inoculants and composted organic wastes are reported as excellent replacements of synthetic fertilizers and fungicides that not only save the soil fertility but also generate positive effect on crop production. Many studies have been done to describe the benefits of organic alterations in improving the important aspects of soil including physical, chemical, and biological but they depend on amount and composition of amendment [20].

In the present study, composted rice husk (CRH) in two quantities (5 and 10 g/2 kg soil/pot) was added on the 7th day of the germination of developing seedlings of sunflower

plants where CRH has proved its potential in growth promotion and improvement in biochemical parameters of plants but effects vary with the microbial treatments involved in composting like rice husk (RH) composted with *T. hamatum* (JUF1), *Bradyrhizobium sp-II* (JUR2), and combination of *T. hamatum* with *Rhizobium sp.* (JUR1 + JUF1) was found effective in improving the shoot and root lengths, total chlorophyll, and biochemical parameters including crude protein and mineral (nitrogen & phosphorus) contents of sunflower plants as compared to control plants treated with uncomposted rice husk (UCRH) and it clearly appeared that the addition of CHR may enhance the organic content of soil which not only help in growth of plants but also make them healthier in term of biochemical parameters. Interestingly, these findings are also compatible to our previous work in which the same JUF1, JUR2 alone, and JUF1 in combination with JUR1 were found effective in the growth of sun flower plants when used as biofertilizers [21]. Rice husk has been used as soil amendment to get better crop yield and also reported to manage plant pathogens including fungi, bacteria, nematodes, and cowpea mottle virus [4]. It was also observed in the present study that composting of rice husk with help of selected microbial treatments improved its total carbohydrate and crude protein contents which could serve as good sources of carbon and nitrogen, respectively, and may facilitate to reestablish or improve the fertility of degraded

TABLE 5: Effect of composted rice husk on physical parameters of *H. annuus* (sunflower) plants.

		Nitrogen %		Phosphorus %	
		30th day	60th day	30th day	60th day
5 g rice husk	Control	1.21 ± 0.12	1.27 ± 0.05	0.07 ± 0.01	0.08 ± 0.00
	JUR1	1.87 ± 0.28 (+54.54)	1.50 ± 0.20 (+18.11)	0.18 ± 0.05 (+157.14)	0.09 ± 0.00 (+12.5)
	JUR2	1.94 ± 0.30 (+60.33)	1.54 ± 0.10 (+21.25)	0.2 ± 0.13 ^d (+185.71)	0.12 ± 0.10 (+50)
	JUF1	4.07 ± 0.84 ^a (+236.36)	1.46 ± 0.13 (+14.96)	0.35 ± 0.42 ^c (+400)	0.09 ± 0.00 (+12.5)
	JUR1 + JUF1	3.27 ± 1.08 ^c (+170.24)	1.49 ± 0.03 (+17.32)	0.12 ± 0.03 (+71.42)	0.09 ± 0.00 (+12.5)
	JUR2 + JUF1	2.0 ± 0.25 (+65.28)	1.52 ± 0.02 (+19.68)	0.15 ± 0.09 (+114.28)	0.11 ± 0.02 (+37.5)
10 g rice husk	Control	1.58 ± 0.1	1.7 ± 0.31	0.07 ± 0.01	0.09 ± 0.05
	JUR1	1.86 ± 0.74 (+17.72)	2.16 ± 0.65 (+27.05)	0.07 ± 0.01 (0)	0.12 ± 0.03 (+33.33)
	JUR2	1.80 ± 0.37 (+13.92)	2.73 ± 0.95 ^d (+60.58)	0.05 ± 0.02 (-28.57)	0.15 ± 0.00 (+66.66)
	JUF1	2.21 ± 0.34 ^d (+39.87)	2.01 ± 0.84 (+18.23)	0.10 ± 0.04 (+42.85)	0.17 ± 0.06 ^d (+88.88)
	JUR1 + JUF1	1.66 ± 0.35 (+5.06)	2.57 ± 0.43 (+51.17)	0.15 ± 0.00 (+114.28)	0.12 ± 0.03 (+33.33)
	JUR2 + JUF1	1.32 ± 0.02 (-16.45)	2.03 ± 0.63 (+19.41)	0.10 ± 0.04 (+42.85)	0.12 ± 0.03 (+33.33)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $P < 0.05$ (LSD). Values within parenthesis represent percent increase or decrease (-) with respective control.

soil. Studies proved that properly processed organic matter or compost can make ample supply of food and energy for native microflora mainly rhizosphere competent one [22].

The experimental microorganisms used in the present study for composting of organic food waste were *T. hamatum*, *Rhizobium*, and *Bradyrhizobium* species, which are well-famous producers of lytic enzymes including β -1,3-glucanase, chitinase, and cellulose [23] which were found efficient in producing biodegradable product from organic waste which might have improved organic matter of soil on its application. This is the first study which describes the utilization of *T. hamatum* in composting of organic food wastes; besides, *T. harzianum* belongs to the same genus, a well-reported fungus utilized in composting procedure and found to be efficient both as alone or in combination with *Rhizobium*, in improving physical properties, organic content of soil, and yield of many nonlegume and legume crops [24]. Beside this, the present study was also focused on recycling organic waste into biodegradable value added product which could be

helpful for sustainable agriculture and environment. Through this work, it has been observed that prepared compost with no living effective microorganism (EM) may also be found favorable for plant growth. However, addition of EM could enhance other characteristics of composts like biocontrol against certain pathogens including better production [25].

5. Conclusion

T. hamatum alone and in combination with *rhizobial* isolates, (JUR1 & JUR2) would be beneficial in the preparation of composted rice husk that may serve as a nutritionally rich biodegradable product that was also found effective in improving the growth and biochemical parameters of sunflower plants possibly by improving the organic content of soil. The composting of organic wastes with the help of microbial inoculants not only helps in recycling of wastes but

also results in the preparation of economical and environmentally friendly organic fertilizer that could provide benefits to agriculture.

Abbreviations

CRH: Composted rice husk
EM: Effective microorganism
LSD: Least significant difference
NPK: Nitrogen phosphorus potassium
RH: Rice husk
S.D: Standard deviation
UCRH: Uncomposted rice husk.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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