Biotechnology Effect of rice husk and chicken manure on highly acidic soils on spinach (*Spinacia Oleracea* L.)

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ABSTRACT

An experiment was carried out in the greenhouse during the 2014 spring season. A complete randomization design was used with three replications. The study aimed to assess the possibility of increasing the growth of spinach grown in highly acidic soil by adding rice husks and chicken waste. Four levels of rice husks and four levels of chicken waste were used. The following results were obtained: Addition of RH15 (g/kg) led to a significant increase in stem diameter, dry and soft weight of spinach leaves, and dry weight of roots. However, when poultry waste was increased to 30, there was a significant increase in plant length, stem diameter, dry and wet weight of leaves, dry and wet weight of roots, while zinc concentration increased significantly when adding 40 rice husk. The interaction between rice husks and poultry waste (0 and 5) led to a significant increase in zinc concentration.

Keywords: Mechanism, Spinach plants, Rice husk, Chicken manure, Growth, Acidic soils

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INTRODUCTION

Husks of rice is a plentiful material produced from the process of peeling rice in many countries of the world, the silica compose approximately 20–30% of its contents. In the countries of underdeveloped, may be as a disposed of rice husk by burned it in the fields. This method practice frequently lead to damage in the environmental, where silica granules remain stuck in the air, accordingly being a possibility result in for respiratory ailments for humans and animals (De Paiva and Rodrigues, 2005).

Rice husks is end output of the harvesting and peeling after maturation for rice, the naturalness gave it as the main source of many course and influential uses (Chris, 2014). Rice husks are a renewable means of fertilizer. The recycle nutrients for the land possible at addition of husk's rice as a fertilize. It can also improve the fecundity of soil, organic matter, physical properties and structure of soil over a wide range of time by using rice husk with manure composts. Rice husk composts are especially useful for increasing of content humic acid in the organic matter of soil, thereby provision a source of nutrients in the soil.

Chicken manure is natural, locally usable and relatively cheap material that the organic vegetable growers can obtain (Michael et al. 2012). Addendum the manures to a lot of soils lead to improvement the physical properties by merge bulky, organic materials and as well as feeding the organisms that endemic in the soil. The organic manures not provide food for the plant only, but they give substances that have the power to act on the non-degradable compounds already in the soil and conversion these compounds to a form that plants can uptake it (Anonymous, 2012). That the orientation to organic planting and the demand of organically produced crops may impact role in reducing chemical residues in food crops (Masarirambi et al., 2010, 2012). Julen et al., (2019) confirmed that adding fresh chicken waste increased the lettuce yield by compare with horse waste that was used in the same study.

Spinach contains a big quantity of minerals and vitamins, essentially calcium and magnesium which are good bone-supportive nutrients; phosphorus; iron; potassium; vitamin A; vitamin K; vitamin B; vitamin E and vitamin C as well as a rich protein source (Lisiewska et al., 2011; Becker et al., 2014; Quartacci et al., 2015). the aim of the study is to evaluation effect of rice husks and chicken wastes on highly acidic soils of spinach.

MATERIALS AND METHODS

A pot experiment was carried out in the plastic greenhouse at the Institute of Sustainable Agrotechnology (INSAT), University Malaysia Perlis in Sungai Chuchuh, Perlis, Malaysia during season of the cultivation of 2014.Factorial experiments according to the Complete Randomized Design (CRD), with three replicates. Three levels of (RH), 5g/kg soil(RH1), 10g/kg soil(RH2)and 15g/kg soil(RH3) with control 0 g/kg soil(RH0) and three levels of (CM), 10 g/kg soil(CM1), 20 g/kg soil(CM2)and 30 g/kg soil(CM3) with control without chicken manure(CM0).Forty-eight pots (20 cm × 35 cm) were filled out with 5 kg dry soil, collected from the layer (0-30cm) of Vang Bintong regions located in Perlis. Table (1) shows the soil farming properties.

The experimental pots were mixed the soil with rice husks and chicken manure. Table (2) presents some of chemical properties of used rice husk and chicken manure. Twenty seeds/pot of Chinese spinach were sown on 10th August (2014), with the same depth. The seedlings were thinned after 15 days of sowing to ten plants per pot. 45 days after sowing of spinach plants; randomly, 5 plants took from each plot; plant height was measured from place the plant of contact with soil surface to the top of leaves. Diameter of the stem was measured by using (Digital Vernier) and at the soil line at the end of the experiment (Makus et al., 1994). The leaves and roots put in paper bags and transfer immediately to the laboratory. Fresh of leaves and roots were weighed before putting the plants in oven for dried at 70 ^{o}C till constant of the weight. Then, measuring of its dry weights (g/plant).

pH (1:10 w/v sample: water extract) using a pH meter with a glass electrode. Total N in the soil were determined according to the methods of Bremner and Mulvany(1982).The Ca, K, Mg and Zn concentrations in the soil samples were measured using (AAS) atomic absorption spectrometer device (AA-7000). The soil sample was dried at 105 ° C. About 3 g of soil was digested with 10 ml of hydrochloric acid and 3.5 ml of nitric acid. The mixtures were left overnight under the running fume cabinet and heated for two hours at 140 ° C the next day. Filtered the mixture using filter paper and then added up to 100 ml of distilled water (Rabah, et al., 2015).

The data were subjected to statistical analysis using the SPSS statistical program (24). The ANOVA contrast analysis table was used to analyze the experimental results of dependent variables (treatments). The least significant difference (LSD) was calculated at $P \leq 0.05$ (Payne et al., 2012).

Table (1): Physical and chemical properties of the used soil during cultivation:

Location	Sand %	Clay %	Silt %	Soil type	pH	EC	N (%)	P mg/kg	Zn mg/kg	Organic matter (%)
Vang Bintong (S1)	64.34	15.07	20.59	Sandy loam	4.18	2.16	1.03 ± 0.02	32.71 ± 1.3	0.49 ± 0.006	0.07

Means are the average of three replicates ± SE.

Table (2): Chemical properties of rice husk and chicken manure:

Type	pH	Moisture content (%)	Ca (%)	Mg (%)	K (%)	N (%)	P (%)	C (%)	Si (%)
Rice husk	6.41	8.74	0.14 ± 0.003	0.048 ± 0.01	0.30 ± 0.04	1.13 ± 0.05	0.03 ± 0.002	33.67 ± 1.72	6.91 ± 0.11
Chicken manure	7.02	53.8	0.16 ± 0.004	0.01 ± 0.001	0.071 ± 0.02	2.10 ± 0.06	0.001 ± 0.000	26.47 ± 1.18	-------

Means are the average of three replicates ± SE.

RESULTSAND DISCUSSION:

Plant height (cm):

Table 3 shows the significant increase in plant height with increase of the rate of adding RH where that the plant height in the RH2 level reached to highest rate22.00cm while the lowest value was 19.42cm in the control treatment RH0, this increase may be due to the improvement of soil properties due to the addition of rice husks, which helps to improved fertility soil and invigoration of the nutrients movement in it (Chris, 2014).Also, the same table indicates to a significant increase in plant height with increased addition rates of CM to the soil, where that the addition of CM3 level led to increased significantly on the rest of the levels reached to 22.75 cm compared with the control of treatment CM0 which it arrived at 17.25cm of plant height, This is due to the increase and availability of nutrients in the soil, the most important of them is the organic matter and nitrogen. These results are consistent with (Michael et al., 2012), where mention that the addition of chicken manure for lettuce crop increased the growth and yield of the plant. Also, Table 3 indicates the presence of interactions between the level of RH2 and level of CM3 surpassed significantly on the rest of interactions in the character of plant height reached24.33 cm. This increase in plant height when using rice husk and chicken waste together, the presence of rice husk in the soil increased the readiness of the organic matter and nutrients contained in chicken waste for movement by improving the physical properties of the soil, which makes the uptake the nutrient elements is easy.

Table (3): Effect of rice husks and chicken manure and its interaction on plant height (cm) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	16.67	17.50	17.67	17.17	**17.25**
CM1	19.17	20.67	21.83	21.33	**20.75**
CM2	20.67	21.33	24.17	23.67	**22.46**
CM3	21.17	22.00	24.33	23.50	**22.75**
Mean of RH	**19.42**	**20.38**	**22.00**	**21.42**	
L. S. D. For CM and RH	**0.318**		**L. S. D. Interaction CM & RH**		**0.637**

-The numbers in the value of L.S.D. It means that there is a significant difference p≤0.05

-"NS" in the value of L.S.D. Means there is no significant difference.

Stem diameter (mm):

Appears in the table 4 that the RH addition to the soil, was there a significant increase in stem diameter of plant with increase of the rate of adding RH as in table 4, where reached the stem diameter to highest rate 3.70 mm in the RH3 level, while the lowest value was 3.25 mm in the control treatment RH0 this is probably due to the high C/N content in the rice husk (Anda et al., 2010), which helps improve soil properties in addition to supplying plants with nitrogen, thus increasing the vegetative system. As well as, the same table indicates to the increased addition rates of CM to the soil led to increase significantly in the plant stem diameter, that the addition of CM3 level superiority of significantly on the rest of the levels where reached to 4.17mm compared with the control of treatment CM0 which it reached to 2.94mm. This increase in stem diameter is most likely caused by the availability of the nutrients necessary for plant growth in the CM, especially N, which is the most important element in plant growth, which enhances plants' cells division, hence, increase growth plant (Kader and Linolberg, 2010; Ilyas et al., 2014).Table 4 mentions to no significant increase in the interactions between the level of RH and level of CM.

Table (4): Effect of rice husks and chicken manure and its interaction on stem diameter (mm) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	2.79	2.87	2.99	3.13	**2.94**
CM1	2.85	2.96	3.09	3.27	**3.04**
CM2	3.43	3.55	3.69	3.98	**3.66**
CM3	3.94	4.08	4.25	4.42	**4.17**
Mean of RH	**3.25**	**3.36**	**3.51**	**3.70**	
L. S. D. For CM and RH	0.113		L. S. D. Interaction CM & RH		NS

-The numbers in the value of L.S.D. It means that there is a significant difference $p \leq 0.05$
-"NS" in the value of L.S.D. Means there is no significant difference.

Fresh weight for leaves (g/plant):

Table 5 shows the significant increase in fresh weight for leaves with increased addition rates of RH to the soil, where that the addition of RH3 level led to increased significantly on the rest of the levels reached to 24.81 g/plant, while arrived at 20.19 g/plant at the treatment RH0. This is probably due to improve soil properties and increased readiness of the main nutrients, which is caused by adding rice husks. This is confirmed by (Anita and Sheo, 2014) who indicated that RH improve the soil properties, the availability of nutrients, which leads to an increase in the yield of *Amaranthuscaudatus*. As for the CM addition to the soil, table 5 has pointed to a significantly enhance of the weight for fresh leaves, where that the fresh weight for leaves in the CM2 level reached to highest value25.41g/plant while the lowest value was 18.98 $g.plant^{-1}$ in the control treatment CM0 this result agree with (Michael et al., 2012) where pointed out to that the use of chicken waste will improve the quality of leafy crops. Same table indicates to a significant increase in interaction between the level of RH3 and level of CM2where gave highest value reached28.73g/plant. This may be due to increased availability of nutrients, such as nitrogen, due to the adding of RH and CM together, which increases the size of the leaves as a result of the division of the cells and thus increase the weight of the leaves. Where the use of RH with CM provides a good and important source of nitrogen, phosphorus, potassium and sulfur (Anonymous, 2008).

Table (5): Effect of rice husks and chicken manure and its interaction on fresh weight of leaves (g/plant) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	15.81	17.70	20.00	22.40	**18.98**
CM1	20.90	23.13	25.91	23.93	**23.47**
CM2	21.93	24.04	26.93	28.73	**25.41**
CM3	22.14	22.80	23.49	24.19	**23.15**
Mean of RH	**20.19**	**21.92**	**24.08**	**24.81**	
L. S. D. For CM and RH	**0.491**		L. S. D. Interaction CM & RH		**0.982**

-The numbers in the value of L.S.D. It means that there is a significant difference $p \leq 0.05$
-"NS" in the value of L.S.D. Means there is no significant difference.

Dry weight for leaves(g/plant):

Table 6 appears that the increased addition rates of RH to the soil led to increase significantly in the dry weight for leaves, 2.91g/plant this was value of addition of RH3 level which superiority of significantly on the rest of the levels of addition of RH, while the control treatment RH0 gave less of value reached 2.02g/plant. This may be due to the improvement of soil acidity due to the addition of rice husks which led to increased movement of nutrients and their readiness in the soil, hence, easily absorption by the plant. This is confirmed by (Boda and Prasad, 2019). This led to accumulation of nutrients in the leaves and increase dry weight. Also, table 6 mention that the CM addition to the soil led to significant increase in dry weight for leaves with increase of the rate of adding CM, where reached the dry weight for leaves to highest value 2.83 g/plant in the CM2 level, while the lowest value was 2.00 g/plant in the control treatment CM0, mostly this increase in the dry weight for leaves was because of increase in the fresh weight for leaves. As well as, the same table indicates to no significantly superior in the interactions between the level of RHand level of CM.

Table (6): Effect of rice husks and chicken manure and its interaction on dry weight of leaves (g/plant) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	1.71	1.89	2.09	2.31	**2.00**
CM1	1.95	2.23	2.54	2.90	**2.40**
CM2	2.11	2.41	2.66	3.03	**2.55**
CM3	2.30	2.62	2.99	3.41	**2.83**
Mean of RH	**2.02**	**2.29**	**2.57**	**2.91**	
L. S. D. For CM and RH	**0.104**		L. S. D. Interaction CM & RH		**NS**

-The numbers in the value of L.S.D. It means that there is a significant difference $p \leq 0.05$
-"NS" in the value of L.S.D. Means there is no significant difference.

Fresh weight for roots (g/plant):

The RH addition to the soil led to a significant effect in this parameter by increasing the quantities of added RH, arriving up to the level RH2 as in table 7, where reached the fresh weight of roots to highest rate 3.86g/plant in the RH2 level, while the lowest value was 2.45 g/plant in the control treatment RH0, the improvement in soil properties perhaps increased the size of the plant, thus, increasing the fresh weight for roots. This is indicated by (XiaoXiao et al., 2018)as the addition of rice husks led to increased porosity, this led to increase the biomass of the root. As well as, the same table indicates to the increased addition rates of CM to the soil led to increase significantly in the plant stem diameter, that the addition of CM3 level superiority of significantly on the rest of the levels where reached to 4.15 g/plant, while the control of treatment CM0 gave the lowest rate 2.40 g/plant. Availability of phosphorus in the soil Table (1) therefore, the addition of CM resulted in the release of phosphorous (Balasubramani et al., 2017), which helped in its absorption and increased root growth(Havlin*et al.*, 2005), thus increasing its size and weight. Table 7 mentions to no significant increase in the interactions between the level of RH and level of CM in the fresh weight for roots.

Table (7): Effect of rice husks and chicken manure and its interaction on fresh weight of roots (g/plant) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	1.71	2.15	2.73	3.01	**2.40**
CM1	2.32	2.92	3.71	3.10	**3.01**
CM2	2.69	3.39	4.31	3.68	**3.52**
CM3	3.09	3.90	4.71	4.90	**4.15**
Mean of RH	**2.45**	**3.09**	**3.86**	**3.67**	
L. S. D. For CM and RH	0.243		L. S. D. Interaction CM & RH		NS

-The numbers in the value of L.S.D. It means that there is a significant difference $p \leq 0.05$
-"NS" in the value of L.S.D. Means there is no significant difference.

Dry weight for roots (g/plant):

Table 8 shows the significant increase in dry weight for roots with increased addition rates of RH to the soil, where that the addition of RH3 level led to increased significantly on the rest of the levels reached to 0.69g/plant comparison with RH0 which it arrived at 0.50g/plant of dry weight for roots. This is due to the fresh weight of the roots due to the increased accumulation of nutrients in it that resulted from adding RH at different levels Table (7).As for the CM addition to the soil, table 8 has pointed to that the increase of the rate of adding CM had a significantly affected in dry weight for roots, where reached at the CM3 level to highest value was 0.70 g/plant, while the lowest value was 0.48 g/plant in the control treatment CM0.This may be due to increased nutrient absorption by the plant after adding CM (Havlin*et al.*, 2005), thus increasing the dry weight of the roots depending on the nutrients stored in it. Same table indicates to no significantly superior in the interactions between the level of RH and level of CM in the fresh weight for roots.

Table (8): Effect of rice husks and chicken manure and its interaction on dry weight of roots (g/plant) of spinach plants under highly acidic soils:

Chicken Manure	Rice husks				Mean of CM
	RH0	RH1	RH2	RH3	
CM0	0.41	0.47	0.53	0.59	**0.50**
CM1	0.47	0.54	0.61	0.68	**0.57**
CM2	0.49	0.56	0.64	0.70	**0.60**
CM3	0.56	0.64	0.73	0.82	**0.69**
Mean of RH	**0.48**	**0.55**	**0.63**	**0.70**	
L. S. D. For CM and RH	0.072		L. S. D. Interaction CM & RH		NS

-The numbers in the value of L.S.D. It means that there is a significant difference $p \leq 0.05$
-"NS" in the value of L.S.D. Means there is no significant difference.

CONCLUSION

Under the highly acidic soil in this study it may be advisable to: the use RH3 of rice husks as well as add or use of CM3 of chicken manure to the presence of a significant increase in all characteristics (plant height, stem diameter, fresh weight of leaves, dry weight of the leaves, fresh weight of roots and dry weight of the roots) compared treatment with control and that may be due to better soil properties and thus increase crop production for spinach. Preferred to use chicken manure and rice husk together because there is significant increase in the most of growth and production characteristics in this study. We also need to do other experiments with the increase in the amount of rice husks because it contributed a lot in improving the properties of highly acidic soils in terms of increased plant growth and production at all additive treatments.

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