

EFFECTS OF MEAT PRODUCT WASTEWATER ON SEED GERMINATION OF RD 41 RICE (*ORYZA SATIVA* L.) FOR REUSE PURPOSE

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ABSTRACT

Meat product wastewater was investigated for the effects on seed germination of RD 41 rice seeds (*Oryza sativa* L.) due to its high quantity and possibility to reuse and mitigate water shortage situation. The wastewater with pH 7.66, EC 722 mS/cm, COD 960 mg/L diluted by deionized water to 0, 12.5, 25, 50, and 100 %, and 10, 20, 30, 40, 50, and 60 % concentrations by volume were applied to germinate 50 rice seeds by a between paper method for 5 days at 5 replicated. The germination percentage, total length, seed vigor index, wet weight/seedling, dry weight/seedling, and the mean germination time (MGT) of radical emergence were investigated. The significantly different of shorter MGT of radical emergence results for 10, 20, and 30 % concentration illustrated the better germination environment, therefore, 30% concentration was suitable to seed germination due to the highest quantity of wastewater to be reused.

KEY WORD: Meat product, Wastewater reuse, Agriculture, Seed germination

INTRODUCTION

Extreme weather event, raising population, expanding agriculture land, and increasing industries caused water shortage situation in many areas. Therefore, reclaiming industrial wastewater for irrigating cereal such as rice (*Oryza sativa* L.) the stable foods, were implemented worldwide. Then there were the researches on applying wastewater such as a pulp industry (Panichsukpatana *et al.*, 2002), the pig manure tea (U-sanggenon *et al.*, 2012), a swine and an aqua culture farm (Hay and Iwai, 2018), an ink industry (Zayne *et al.*, 2015), sugar mill (Rath *et al.*, 2013, Saini and Pant, 2014; Nagda *et al.*, 2006, tannery (Faiz *et al.*, 2010) and seafood processing (Mendez and Joseph, 2014) on rice, barley, wheat, and quinoa germination.

At the germination stage, exogenous and endogenous factors regulated seed germination were water, temperature, light, and phytohormones

(He and Yang, 2013). Previous researches stated that factors affected seed germination were salinity, micro nutrients such as Ca, Cu, Zn, Fe, Na, non-nutrients such as Hg, Pb, Cd, and Cl. NaCl and other salts produced salt stress, interrupted osmotic relations of water and seeds, enhanced conductivity of solutes that seed adsorbed for germination, inhibited water uptake, cell expansion and lateral bud development in root medium, and reduced % seed germination, protein, starch content, and biomass production (Yeo and Flower, 1986; Glenn and Brown, 1999; Munns and Tester, 2008; Rajakumar *et al.*, 2013; Mendez and Joseph, 2014). Moreover, Na⁺ accumulation in cells caused decreasing cellular metabolism activity including photosynthesis, necrosis, leaf mortality, and chlorosis. However, some salt solution showed beneficial result on seed germination, Theerakulpisul *et al.* (2016)

found that among NaCl, KCl, CaCl, and KNO₃

seed priming agents under salt stress 150 mM NaCl, the 0.75 % KNO₃ reduced proline content, the symptom of salt stress, by functioning as an osmoregulatory and essential elements for metabolic process, and prevented salinity building up in plant tissue (Shabala and Cuin, 2008, *Szczerba et al.*, 2009). Dutta and Boishya (2000) investigated that higher concentration negative effects occurred from the surplus of TN, PO₄, K, SO₄, Cl that injured plant growth from decrease water absorption and obstruct the metabolic processes, moreover high Ca and Mg could cause injury to plant and had negative results on seedling growth (Rajannan and Oblisami, 1979). High amount of Na, Cl, SO₄ could obstruct the uptake of other nutrient such as K, Ca, P and Mg and caused shoot length reduction. High sodium content could reduce water intake rate and increase alkalinity. There were many researches applied industrial wastewater on seed germination. The treated effluent from ink industry with pH 7.25, COD at 388 mg/L, Na at 127 mg/L, Cu at 0 mg/L was applied on barley seed germination (Zayneet al., 2015) and yield 99 % germination. Swine effluent at 100 % concentration with high EC at 3.801 ms/cm, high COD at 7,200 mg/L gave 0% germination (Hay and Iwai, 2018). The swine manure tea was also applied as seed priming for rice seed germination, 5% concentration by volume increased seed vigor in term of increase seed quality enhancement (U-sanggenon et al., 2020). Sugar factory distillery spent wash and sugar mill effluent with concentration less than 25% concentration gave 80-99.6 % germination for wheat and rice while 50-100% concentration yielded 25-58% germination (Rath et al., 2013, and Samuel and Muthukkaruppan, 2011). Saini and Pant (2014) applied paper mill effluent with pH at 8.4, EC 4.27 mS/cm, COD 590 mg/L on rice germination in soil and found that at 10-30 % concentration gave 91-99 % germination.

The purpose of this study is to reveal characterization of meat product wastewater and its effect on the germination and seedling growth results and to investigate the suitable concentration on RD 41 rice seed germination.

MATERIALS AND METHODS

Collection of wastewaters

Meat product wastewater was collected from a Sausage and beacon factory in Muel Sap Land Factory Land, Khok Krabue, Mueang Samut Sakhon

District, Samut Sakhon Province, Thailand and stored in plastic containers and keep in refrigerator below 5 °C until application.

Collection of seed

RD 41 seeds were supported by the Pathum thani Rice Research Center, Thailand.

Analytical Procedure

Wastewater was analyzed as following; pH, Temperature, Electrical Conductivity (EC), Dissolved Oxygen (DO), Biochemical Oxygen demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solid (TDS), Alkalinity, Fats, Oil and Grease (FOG), Total Kjeldahl Nitrogen (TKN), Ammonia Nitrogen (NH₄), Nitrate (NO₃), Phosphate (PO₄), Potassium (K), Sulphate (SO₄), Calcium (Ca), Magnesium (Mg), Iron (Fe), Boron (B), Copper (Cu), Zinc (Zn), Arsenic (As), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg) according to AWWA (2017). Sodium Adsorption Ratio (SAR) was calculated by the following equation:

$$SAR = \frac{Na^+}{\left[\frac{Ca^{2+} + Mg^{2+}}{2} \right]^{1/2}}$$

Where,

SAR = Sodium Adsorption Ratio

Na⁺ = sodium concentrations (meq/L)

Ca²⁺ = calcium concentrations (meq/L)

Mg²⁺ = magnesium concentrations (meq/L)

Experimental Design

Seed germination experiments were conducted for 5 days at a Laboratory, The King's Royally Initiated Laem Phak Bia Environmental Research and Development Project, Phetchaburi Province, Thailand. The experiment conducted by a Completely Randomized design (CRD) with five replications. RD 41 rice seeds were randomly selected for 50 seeds and germinated by between paper method using 25 cm. x 30 cm. germination paper in 9 in x 14 in plastic bags, covering loosely with rubber bands, moistening with 30 mL of 0, 12.5, 25, 50, 100 % concentration respectively, keeping at room temperature approximately 35 °C and provide light with fluorescent bulb 24 hrs. After 5 days % germination, root length, shoot length, seedling vigor index, fresh weight, and dry weight were investigated. Then the results of 100% concentration showed significant difference (P ≤ 0.05) compared to

0, 12.5, 25, 50 % for seed vigor index, and dry weight. The 0 - 60% concentration with 10% interval were applied to germinate the seed and the mean germination time (MGT) of radical emergence was additionally examined.

Germination Percentage (GP)

Germination percentage calculated from number of seeds germinated per total number of seeds by the following equation:

$$GP = \left(\frac{N}{N1} \right) 100$$

Where,

GP = Germination Percentage

N = The number of germinated seeds

N1 = Number of seeds used

Seedling Length

The seedling length was the total length of the root length and shoot length. The root length was measured by the primary seed radical and the shoot length was measured from the primary seed plumule in centimeter.

Fresh Weight and Dry Weight

The electronic balance was used to determine fresh weight seedling after the germination and dry weight seedling after drying in the hot air oven at 70°C for 72 hrs.

Vigor Index (VI)

Root length and shoot length of seedling and percent germination were calculated for Seed Vigor Index by the following equation:

$$SVI = (RL + SL) \times GP$$

Where,

SVI = Seed Vigor Index (cm.)

RL = Mean Root length (cm.)

SL = Mean Shoot length (cm.)

GP = Germination Percentage

Mean germination time (MGT) of radical emergence (day)

The Radical emergence mean germination time was calculated by the following equation:

$$MGT = \frac{\sum(nT)}{\sum n}$$

Where,

n = number of seeds newly germinated (2 mm, radicle emergence) at time T

T = hours from the beginning of the germination test

\sum_n = final germination

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) Version 10 was applied to analyze the data including the analysis of variance (One Way ANOVA). The results mean was compared with the least significance difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Meat product wastewater contained *E. coli* at 2×10^5 MPN/100 mL while FAO's agricultural reuse in crops that are consumed and not processed commercially identify the criterion after secondary disinfection at < 200 MPN/100mL. Total Coliform Bacteria and the Fecal Coliform Bacteria were observed and found at $2,400 \times 10^3$ MPN/100mL and $1,300 \times 10^3$ MPN/100 mL respectively. The bacteria were found due to the pipe leakage from the restroom to the wastewater collection tank before transferring to the central wastewater treatment of the factory land. The challenges on pathogens to reuse wastewater for agriculture reuse also found in the developing countries (Jaramillo and Restrepo, 2017), however in this case the pipe fixation will solve the contamination.

The physical and chemical characteristics of meat product wastewater were illustrated and compared to the guideline for agriculture reuse (FAO, 1985), and Standards and Meteorology Corporation Jordan (WHO, 2005) were compared in Table 1. The value of potential irrigation problem parameters such as electrical conductivity (EC), TDS, SAR, Na, Cl, B, NO_3 , pH were 0.722 dS/m, 484 mg/L, 14.08, including 388.66, 479.64, 0.0419, < 0.5 , mg/L and 7.66 respectively, observing that salinity in term of EC and TDS were identified as slight-moderate degree of restriction on use, however SAR, Na, and Cl value was identified as sever degree of restriction on use. The value for parameters respected to the wastewater guideline for agricultural reuse such as As, Cd, Cr, Cu, Fe, Mn, Pb, Zn, BOD, TSS were < 0.002 , < 0.0005 , 0.0031, 0.0075, 0.3290, 0.0121, 0.001, 0.2228, 276 and 81 mg/L consecutively, noticing that only BOD that exceeding the guideline value due to the wastewater was raw water before treatment. The value of parameters the maximum permissible value for field crops and forestry by the Jordanian Standard such as COD, PO_4 , SO_4 , HCO_3 , Mg, Ca, FOG were 960, 5.38, 259.30, 225.01, 13.87, 34.91, 34.06 mg/L consecutively, perceiving that COD was exceeding the recommended value. K was additionally investigated with the value of 53.98

mg/L. Some physical and chemical wastewater quality were exceeding the recommended value according the collection before the wastewater treatment plant, however this situation was also observed by Adesuyi *et al.*,(2016).

The germination %, seedling length, seed vigor index, wet weight/seedling, and dry weight/seedling results from 0, 12.5, 25, 50, 100 %

wastewater concentration were shown in the Table 2. The germination %, seedling length were highest at 12.5% with the value of 97.20 ± 2.28 % and 15.6 ± 1.3 cm while the germination % and seedling length were lowest at 100% wastewater concentration with the value of 94.80 ± 3.03 % and 13.4 ± 1.6 cm. From 25 % wastewater concentration and other higher wastewater concentration the germination % and

Table 1. Wastewater physical and chemical quality

Parameters	Meat Product Wastewater	Degree of restriction on use ¹			Max. permissible value ²		
		None	Slight-moderate	Severe			
Potential Irrigation Problems							
Salinity							
	EC (dS/m)	0.722	<0.7	0.7-3.0	>3.0	-	
	TDS (mg/L)	484	<450	450-2,000	>2,000	1500	
Infiltration							
SAR	0-3	EC	>0.7	0.7-0.2	<0.2	9.0	
	3-6		>1.2	1.2-0.3	<0.3		
	6-12		>1.9	1.9-0.5	<0.5		
	12-20	(SAR 14.08, EC 0.722)	>2.9	2.9-1.35	<1.3		
	20-40		>5.0	5.0-2.9	<2.9		
Specific Ion Toxicity (affects sensitive crops)							
SAR	Na (mg/L)	388.66	<3.0	3.0-9.0	>9.0	230	
	Surface Irrigation		<69	>69	-		
	Cl (mg/L)	479.64	<142	142.0-355.0	>355		400
	B (mg/L)	0.0419	<0.7	0.7-3	>3.0		1.0
	NO ₃ (mg/L)	<0.5	<5.0	5.0-30	>30		45
	pH	7.66		6.5-8.4			6-9
Others							
	As (mg/L)	<0.002		0.1	0.1		
	Cd (mg/L)	<0.0005		0.1	0.01		
	Cr (mg/L)	0.0031		0.1	0.1		
	Cu (mg/L)	0.0075		0.2	0.2		
	Fe (mg/L)	0.3290		5.0	5.0		
	Mn (mg/L)	0.0121		0.20	0.2		
	Pb (mg/L)	0.0010		5.0	5.0		
	Zn (mg/L)	0.2228		2.0	5.0		
	BOD (mg/L)	276		30**	300		
	TSS (mg/L)	81		260**	150		
	COD (mg/L)	960		-	500		
	PO ₄ (mg/L)	5.38		-	30		
	SO ₄ (mg/L)	259.30		-	500		
	HCO ₃ (mg/L)	225.01		-	400		
	Mg (mg/L)	13.87		-	100		
	Ca (mg/L)	34.91		-	230		
	FOG	34.06		-	8.0		
	K*(mg/L)	53.98		-	-		

¹FAO's water quality for irrigation (FAO,1985)

²Jordanian Standard (JS: 893/2002) for effluent reuse guideline of maximum permissible value for field crops and forestry (WHO, 2005) *K was additional observed due to its function as macronutrient for plants, Al, Be, Co, F, Li, Mo, Ni, Se, V were specified²but were not investigated due to the low possibility to found from the factory processes and laboratory limitation ** FaO's agricultural reuse in crops that are consumed and not processed commercially

seedlings length were gradually declined. However, the wet weight/seedling and the dry weight/seedling were not significantly different from 0% wastewater concentration or control concentration. Moreover, the seed vigor index was highest at 12.5% wastewater concentration at the value of 1513.5 ± 155.5 and not significantly different with the seed vigor index results from 25% and 50% wastewater concentration as shown in Fig 1. The observation that the higher concentration caused lower % germination, which conformity with the study of Rani and Alikhan (2007). However, to identify the most suitable wastewater concentration, germination the seed with the 0 - 60 % wastewater concentration with 10% interval were investigated with additional radical emergence mean germination time (MGT) parameter.

The germination %, seedling length, seed vigor index, wet weight/seedling, dry weight/seedling, and radical emergence (MGT) results from 0, 10, 20, 30, 40, 50, 60 % wastewater concentration were shown in the Table 3. The germination %, seed vigor index, wet weight/seedling, and dry weight/seedling results were not significantly different. However, the seedling length result for 60 % wastewater concentration were significantly different from 0, 10, 20, 30, 40, and 50 % wastewater concentration with the value of 1218.2 ± 350.1 cm

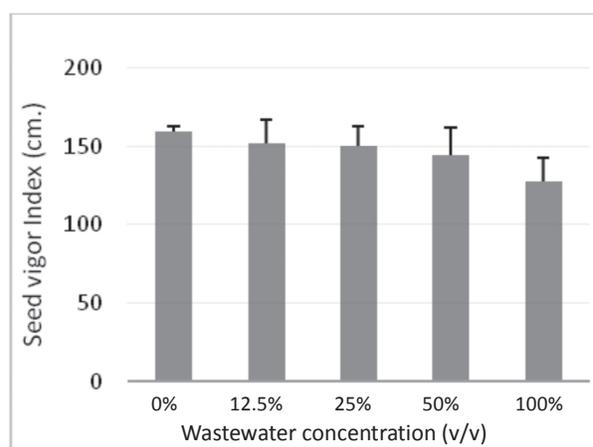


Fig. 1. Seed Vigor Index for 0, 12.5, 25, 50 % wastewater concentration

radical emergence (MGT) results were not significantly different among 0, 10, 20, and 30% wastewater concentration with the value of 1.22 ± 0.07 , 1.21 ± 0.17 , 1.27 ± 0.11 and 1.34 ± 0.07 day respectively, but significantly different with 40, 50 and 60% wastewater concentration with the value of 1.68 ± 0.07 , 1.63 ± 0.09 , and 1.84 ± 0.02 day respectively as shown in Fig 2.

The higher concentration or higher dissolved solid, which contributed to salinity, inhibited germination and seedling growth due to it disturbed

Table 2. Effect of 0 - 100 % concentration(v/v) wastewater on RD 41 seed germination

Wastewater Concentration v/v (%)	Germination Percent (%)	Seedling length (cm)	Seed Vigor Index	Wet weight/ seedling (mg.)	Dry weight/ seedling (mg)
0	97.20 ± 1.10^a	16.3 ± 0.4^a	$1,588.5 \pm 37.6^a$	60.85 ± 3.44^a	16.73 ± 0.43^a
12.5	97.20 ± 2.28^a	15.6 ± 1.3^a	$1,513.5 \pm 155.5^a$	57.43 ± 4.48^a	17.59 ± 0.60^a
25	95.20 ± 3.03^a	15.5 ± 1.9^a	$1,500.6 \pm 129.2^a$	59.75 ± 5.75^a	17.70 ± 0.65^a
50	96.40 ± 2.61^a	14.9 ± 1.7^a	$1,438.4 \pm 180.8^{ab}$	63.03 ± 4.31^a	17.58 ± 0.59^a
100	94.80 ± 3.03^a	13.4 ± 1.6^a	$1,276.1 \pm 146.6^b$	58.39 ± 4.28^a	17.69 ± 0.24^a

Table 3. Effect of 0 - 60 % concentration (v/v) wastewater on RD 41 seed germination

Wastewater Concentration v/v (%)	Germination Percent (%)	Seedling length (cm)	Seed Vigor Test		Wet weight/ seedling (mg)	Dry weight/ seedling (mg)
			Seed Vigor Index (cm)	Radical emergence MGT (day)		
0	98.00 ± 2.83^a	14.10 ± 2.39^a	$1,401.2 \pm 216.9^a$	1.22 ± 0.07^a	57.20 ± 4.65^a	19.30 ± 0.50^a
10	98.80 ± 1.79^a	14.17 ± 2.66^a	$1,399.8 \pm 263.2^a$	1.21 ± 0.17^a	55.20 ± 1.26^a	18.40 ± 0.55^a
20	96.50 ± 2.52^a	14.92 ± 3.07^a	$1,393.6 \pm 249.3^a$	1.27 ± 0.11^a	56.30 ± 1.26^a	18.00 ± 0.12^a
30	96.80 ± 2.28^a	14.29 ± 2.06^a	$1,335.8 \pm 134.9^a$	1.34 ± 0.07^a	55.80 ± 1.92^a	17.00 ± 2.83^a
40	96.80 ± 1.79^a	14.39 ± 2.45^a	$1,332.6 \pm 168.8^a$	1.68 ± 0.07^b	54.40 ± 2.97^a	18.60 ± 0.55^a
50	98.40 ± 0.89^a	14.86 ± 2.21^a	$1,336.3 \pm 215.4^a$	1.63 ± 0.09^b	57.20 ± 1.79^a	16.40 ± 4.72^a
60	97.00 ± 2.00^a	12.56 ± 3.61^b	$1,218.2 \pm 350.1^b$	1.84 ± 0.02^c	54.00 ± 2.71^a	19.30 ± 0.50^a

osmotic relationship of the seed and water, caused less water absorption, reduced amount of oxygen, restricted the energy supply, slowed the growth and

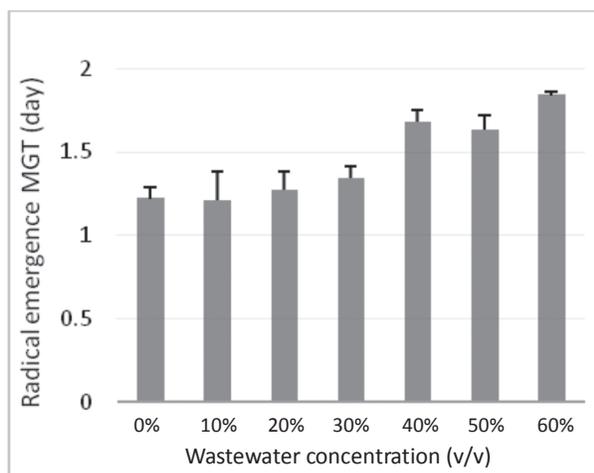


Fig. 2. Radical emergence MGT for 0, 12.5, 25, 50% wastewater concentration

seedling development, and obstructed the metabolic processes (Dutta and Boishya, 2000, Hussain *et al.*, 2013). Higher FOG content was suspected to reduced seed germination, but Hay and Iwai, (2017) study results revealed that FOG from aquaculture wastewater with the value of 1,500 mg/L also yield 95% germination. Higher Ca and Mg content could cause injury to plant and had negative results on seedling growth (Rajannan and Oblisami, 1979). Higher Na, Cl, SO₄ content could obstruct the uptake of other nutrient such as K, Ca, P and Mg and caused shoot length reduction. Higher sodium content could reduce water intake rate.

At lower concentrations P, and K, were in the optimize for metabolic enzyme to catalyze germination process and enhance seedling growth (Bam *et al.*, 2006). Moreover, at lower concentrations, the negative effect from excess nutrients such as Fe on cell membranes, DNA, and protein damage from free radical (Arora *et al.*, 2002, Rasafi *et al.*, 2016), Pb on morphology, growth and photosynthetic process (Bot *et al.*, 2008, Asati *et al.*, 2016), Cu on osmotic potential and water flow to the seed (Quartacci *et al.*, 2001), Cr on cell membrane damage (Asati *et al.*, 2016), Zn on reduce root length and shoot length (Peralta *et al.*, 2001, Rasafi *et al.*, 2016) were lower. Additionally, K, PO₄, Ca Clin the wastewater could form KH₂PO₄, KCl, CaCl₂ and function as seed priming agent that 1) mitigated salt stress, decreased the electrolyte leakage, suppressed Na uptake and stimulating K uptake in shoot (Iqbal *et al.*, 2006,

Theerakulpisut *et al.*, 2017), 2) ameliorated α-amylase activity, which enhanced the level of soluble sugars in the primed Kernels (Farooq *et al.*, 2006) 3) shortened the seed germination time, increased dehydrogenase activity and accelerate catabolic chemical in anaerobic respiration during germination (Oaikhena *et al.*, 2013)

The radical emergence MGT results was the key to justify the suitable wastewater concentration for seed germination and rice vigor test (Onwimol *et al.*, 2015; U-sanggenon *et al.*, 2020) due to the radical emergence MGT reflected deterioration of seed quality the among the non-significantly different result of radical emergence MGT from 10, 20, 30% wastewater concentration, 30% concentration is suitable for seed germination practice.

CONCLUSION

The study results revealed that meat product wastewater that contained numerous nutrients at higher concentration showed some negative effect on seed germination however at lower concentration fewer negative effects were found and suitable dilution could be practically apply to germinate rice.

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