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Influence of Herbicides-pyroligneous Acids Mixtures on Some Soil Properties, Growth and Grain Quality of Paddy Rice

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Abstract

The influence of herbicides-pyroligneous acids mixtures on soil chemical properties, growth and grain quality of paddy rice (Japonica cv. *Ilpum)* was evaluated in a field experiment. There were thirteen treatments including control (hand weeding), 100% and 50% application rate of three herbicides: Azimsulfuron+cafenstrole (AC), Mefenacet+pyrazosulfuron-ethyl (MPE) and Betazone+cyhalofop-butyl (BCB) and 6 combination of three types of herbicides at 50% recommended rate (RR) diluted with two types of pyroligneous acids: wood vinegar (WV) and rice vinegar (RV) at 500× dilutions; laid out in randomized complete block design with four replications. Results showed that lower application rate of herbicides combined with WV or RV gave a comparable effect in controlling weeds and on plant growth and chlorophyll content with their respective 100% RR application rate. Among different treatments, 50% AC + WV increased the number of spikelet per panicle and improved the grain quality compared to 100% AC. Treatment 50%MPE+RV decreased the spikelet number per panicle while 50% BCB+WV also reduced spikelet number per panicle, total protein content, and whole grain yield. After harvest, soil acidity, organic matter (OM), available P and EC increased due to treatments application. Overall, depending on the herbicide type, mixing 50% RR of herbicides with 500× of RV or WV dilutions can effectively control the weeds in paddy rice such as *Echinochloa crusgalli*, *Monochoria vaginalis* and *Scirpus juncoides* without suppressing the growth, and yield of rice with acceptable soil impacts that promotes environment health. © 2015 Friends Science Publishers

Keywords: Herbicides-pyroligneous mixture; Residual soil nutrients; Rice grain quality; Rice vinegar; Wood vinegar

Introduction

Sustaining productivity in conventional agriculture entails high quantity of agricultural inputs such as chemical fertilizers and pesticides. In fact, to augment yield in crop production and meet the current demand, there is unprecedented increase in varieties and consumption of pesticides worldwide (Carvalho, 2006). These production practices have resulted to pesticide misuses, heavy environmental pollution and health risks. To safeguard human health, threatened species and ecosystems from pesticide pollution, the consumption and pollution of pesticides worldwide should be minimized by looking alternative sources which are less harmful to the environment and ecosystems (Zhang *et al*., 2011). Ideally, environment friendly pesticides should be highly efficient with high biological activity that may greatly reduce pesticide uses and pollution to the environment, be non-toxic and causes no or minimal pollution (Yang *et al*., 2007; Zhang *et al*., 2011).

Among the different pesticides, herbicides consumption increased rapidly, from 20% in 1960 to 48% in 2005 of the total world pesticide consumption (Zhang *et al*., 2011). According to report, the proportion of insecticides and fungicides/bactericides consumption declined and there was rapid increase of herbicide consumption alongside with enhanced agricultural intensification and productivity. Therefore, indiscriminate use of herbicides in agricultural production systems occurred and ought to be a major contributor to environmental degradation (Arias-Estévez *et al*., 2008; Zhang *et al*., 2011).

Concurrently, various agricultural policies and management programs demand to minimize environment and health hazards from continuous use of pesticides and other synthetic chemicals in controlling pests thus leading farmers to look for alternative plant protection techniques. One of the natural products that have been rapidly gaining attention is the by-product of charcoal production, pyroligneous acids, brown transparent liquid produced by condensation of smoke from process of producing charcoal. Examples of pyroligneous acids are wood and rice vinegar traditionally used as pesticide especially in countries, where the price of synthetic chemicals is too high for small scale

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farmers (Qaim and De Janvry, 2005). Aside from their use as a pesticide, wood and rice vinegar is also known as fungicide, repellent and insecticide, herbicide, and plant growth enhancer (Tiilikkala *et al*., 2010). According to Yoshimoto (1994), the main components of pyroligneous acids are methanol and acetic acid among more than its 200 compounds reported earlier. Yoshimoto (1994) reported the positive influence of wood vinegar on crop growth and germination. Chalermsan and Peerapan (2010) found that wood vinegar concentration less than one percent can activate plant growth and it may also reduce pathogenic microorganisms and soil arthropods when sprayed on the ground. Kang *et al*. (2008) reported that mixing wood vinegar with carbon-based soil amendments combined with chemical fertilizers improved the yield and grain quality of rice compared to chemical treatments alone. Similarly, Masum *et al*. (2013) showed that combination of wood vinegar and 50% RR of chemical fertilizer improved the yield of *T. aman* rice while wood vinegar applied to the soil surface increased the population of beneficial microbes and promote plant root growth (Tancho, 2008).

Recently, Acenas *et al*. (2013) studied the efficacy of wood and rice vinegar (a pyroligneous acid from rice hull) against barnyardgrass (*Echinochloa crus-gali*) under greenhouse conditions. They showed the potential of mixing WV or RV with herbicide to reduce the application rate and control barnyardgrass especially the mixture of WV or RV with BCB (bentazone + cyhalof-butyl). Cueto (2012) showed that seedling height reduction of *Echinochloa crus-gali* was not significantly different when using 100% rate of herbicide and 50%+pyroligneous acids. More concentrated dilutions of either wood vinegar (WV) or rice vinegar (RV) resulted in greater seedling height reduction of *E. crus-gali* than less concentrated dilutions. Similar studies also revealed that in terms of protein content, reduced application rate and mixing with RV or WV resulted in low protein synthesis comparable to full application rate of herbicide (Acenas *et al*., 2013; Cueto, 2012). However, in order to determine the overall efficacy of herbicide-pyroligneous mixture for farmers' adoption, there is also need to assess its impact to soil properties and subsequent effect on plants capability to overcome stresses brought about by herbicides application. Apart from increasing the efficacy of herbicides against target weeds, application of pyroligneous acids may influence soil properties and nutrient availability that would subsequently affect plant growth and quality of products. In present study, we evaluated the potential of herbicides-pyroligneous acid in controlling weeds in paddy field and determine its effect on yield and grain quality of rice and its potential effects on some soil chemical properties after crop harvest.

Materials and Methods

Site Description

A field experiment was conducted at Gyeongsangbuk-do Agricultural Research and Extension Services (35°57'17''N and 128°33'39''E) located in Daegu, South Korea. The soil was characterized moderately acidic, containing moderate to high levels of organic matter and high amounts of exchangeable Ca (Table 1).

Treatment Application and Crop Establishment

The experiment was laid out in completely randomized block design with thirteen treatment combinations of herbicides-pyroligneous acid mixture with four replications and sub-plots of 5 m × 4 m (20 m^2). The post emergence herbicides were Azimsulfuron+cafenstrole (AC), Mefenacet+pyrazosulfuron-ethyl (MPE) and Betazone+cyhalofop-butyl (BCB) and the pyroligneous acids included rice vinegar (RV) and wood vinegar (WV). AC and MPE were granular powder while the BCB, a liquid herbicide. Pyroligneous acids from wood (*Quercus sp.*) and rice husk/hull were obtained from the Korean Forest Research Institute (KFRI) and Daewon Global System Integration (Daewon GSI), respectively. The physical and chemical properties of WV and RV are presented in Table 1. The treatments included were 100% RR of herbicide, 50% RR of herbicides, 50% RR herbicide + RV500× dilution, 50% RR herbicides + WV500× dilution and non-treated (NT) control.

Rice seeds were pre-germinated for 48 h in tap water in dark at 30°C and germinated seeds were sown in a seedling tray (58 cm × 28 cm × 3 cm). Thirteen days old seedlings were machine-transplanted with planting distance of 30 cm × 14 cm. Other management practices including fertilizers recommended by RDA in rice cultivation were employed. The basal fertilizers applied were 55 kg N ha^{-1} (Urea), 45 kg P_2O_5 ha^{-1} (Super phosphate) and 40 kg K_2O ha^{-1} (Potassium chloride). At tillering and panicle initiation stages, urea was applied at 22 kg N ha^{-1} and 33 kg N ha^{-1}, respectively.

At 15 DAT, the AC and MPE, granular herbicides evenly and diluted pyroligneous acids were applied to soil. The BCB, a liquid herbicide was applied 25 DAT by spraying based on the manufacturer's recommended rate and methods of application followed by application of diluted pyroligneous acids into the soil. Acids at 500× dilution were applied at 10 L per 1000 m^2.

Measurement of Efficacy of Herbicide-pyroligneous Acids

Weeds were sampled from unit area (1 m^2) of each plot 30 days after treatment application. All weeds were collected, identified, and classified to either annuals or perennials. Weed samples were washed thoroughly and then fresh and dry weight was recorded after 3 days of oven drying at 70°C. Herbicidal efficacy for each weed species in each treatment was calculated by dividing the dry matter yield of weeds with the dry matter yield of weeds of the control multiplied by 100.

Measurement of Growth Characteristics and Yield Components

Growth characters (plants height, tiller number, and chlorophyll content), yield components (panicle number, panicle length, spikelets per panicle, ripening ratio, and 1000-grain weight), and paddy yield was measured. Growth characters were measured at maximum tillering (MTS) and heading stage (HS).

At harvest, plants in 100 randomly selected hills were processed for the analysis of grain quality. Dehulled and polished grains were analyzed for percent whole grain, broken grain, damaged grain and chalkiness using a Whole Grain Analyzer (Cervitec 1625 Grain Inspector, Foss Tecator, Sweden). Protein and amylose contents (%) were analyzed using a Grain Value Analyzer (Foss Infratec 1241 Grain Analyzer, Sweden) while palatability was measured in 30 g-portion of milled rice per treatment using a TOYO-taste meter (TOYO MB-90A, Japan).

Nutritional Quality Analysis

Rice starch lipids, known to exist as inclusion complexes with amylose in starch granules, were measured according to the method of Takahashi and Seib (1988) with a slight modification. Two grams (2 g) of brown rice sample was placed into a centrifuge tube, and then 25 mL of 75% *n*-propanol solution was added. The mixture was boiled in a water bath for 2 h at 100°C. Extracted lipids were saponified by the method of Ruibal-Mendieta *et al.* (2004), and the methyl esters of fatty acids were extracted with *n*-hexane for analysis. The gas chromatograph (6890 plus, Hewlett Packard Co. Palo Alto, CA, USA) was equipped with a DB-225 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) coupled to a mass spectrometer (JMS700, JEOL, Tokyo, Japan). The column was held at 140°C for 1 min, then programmed to 200°C at 5°C per min and increased to 10°C per min to 230°C, and finally held for 10 min. Helium was used as the carrier gas at a flow rate of 1 mL per min (ionizing energy; 70 eV). Fatty acid analysis was done following the procedure of Ruibal-Mendieta *et al.* (2004). Brown rice sample was methylated by reacting with methanolic potassium hydroxide and analyzed for fatty acid using GC-MS (GC-MS: HP 6890 series, Hewlett Packard, Japan) equipped with a DB-225 column (30 m × 0.25 mm × 0.25 μm). The injector temperature was 250°C, while the column temperature was 140°C for 2 min and gradually increased to 200°C at 5°C per min and finally increased to 220°C at 10°C per min. The injection volume was 1 μL injected at a flow rate of 1 mL per min.

For the amylose contents, analysis was done by adding 2 N NaOH to the sample and then neutralized by adding 0.11 N acetic acid followed by the addition of iodine-potassium iodine reagent (1% I_2 in 10% KI). Absorbance of the samples was scanned using a UV/visible spectrometer (DU 800 series, Beckman Coulter Inc., Fullerton, CA, USA). The amylose contents of rice starch were determined as the absorbance at 680 nm based on the standard curve of a pure amylose (Sigma, St. Louis, MO, USA).

Table 1: Physical and chemical components of pyroligneous acids and soil properties before the experiments

Items	Wood Vinegar[1]	Rice Hull Vinegar[1]	Soil[2]
pH	3.03	1.86	5.50
Amount of acid (%)	3.75	3.60	
Acetic acid (%)	3.52	3.33	
Propionic acid (%)	0.23	0.27	
Sediment (%)	0.05	0.06	
Brix (%)	3.5	-	
Organic matter:N ratio	-	0.42	
Transparency	Transparent	Transparent	
Color	Reddish Brown	Golden Yellow	
EC (dS m^{-1})			0.47
OM (g kg^{-1})			24.97
Total Nitrogen (g kg^{-1})			0.22
Available P_2O_5 (mg kg^{-1})			72.3
Exch. Bases ($cmol^+$ kg^{-1})			
K			3.38
Ca			1.37
Mg			0.47
Available SiO_2 (mg kg^{-1})			65.67

[1]Source: A & F Research Co., Ltd
[2]Data are means of analysis of 3 replicates

Soil Sampling and Chemical Analysis

After harvest, five sub-samples in each plot were collected and combined to make a representative sample. Soil samples were air-dried, sieved to pass through 2 mm, and analyzed for chemical properties and nutrient concentrations. Soil pH and electrical conductivity (EC, dS m^{-1}) were measured in 1:5 soil-water ratio. Cation exchange capacity (CEC, $cmol^+$ kg^{-1}) was measured using ammonium acetate (pH=7) and organic matter content (OM, %) using the Tyurin IV method. Total nitrogen (Total N, %) was determined through Kjeldahl digestion and distillation, available phosphorus (P_2O_5, mg kg^{-1}) by Lancaster P extraction method, and exchangeable potassium (Exch. K, $cmol^+$ kg^{-1}) was detected through Flame Type - Atomic Absorption Spectrophotometry (AAS) after extraction with 1 N ammonium acetate at pH 7. Exchangeable calcium (Ca) and magnesium (Mg) was determined after EDTA-titration, and available silica (SiO_2, mg kg^{-1}) in soil through spectrophotometric methods. Each treatment consisted of four sample replicates.

Statistical Analysis

Data were statistically analyzed for significance by the GLM procedure using SAS. Fisher's least significant tests were conducted to compare treatment means when ANOVA showed a significant level for differences among treatments.

Results

Herbicidal Efficacy of Herbicides-pyroligneous Acid Mixtures

The weeds identified in experimental plots were *Echinocloa crus-gali* and *Monochoria vaginallis* belong to annual weeds and *Scirpus juncoides* for the perennial weeds (Table 2). All herbicides treatments alone or in combination with WV or RV effectively reduced the growth of *E. crus-galli* except for the treatment involving BCB 50% and 100% unable to fully eradicate *S. juncoides*. In contrast, all the herbicides-pyroligneous treatments could not totally eradicate *M. vaginalis* but dry matter yield of *M. vaginalis* not varied significantly among treated plots.

Vegetative Growth

At maximum tillering stage, no significant difference was observed for the different herbicide-pyroligneous mixture. On the other hand, the chlorophyll content were generally higher in treatments with herbicides AC and MPE and its mixture with RV or WV compared to control (Table 3). In BCB applied plants, 50% BCB + WV had higher SPAD value compared to the control. At heading stage, plant height and SPAD value were significantly affected by different treatments except for tiller number. Except for 50% AC + RV and 50% MPE + RV, which were taller and all other treatments had plant height comparable to control. However, the chlorophyll contents in most of the treatments were higher than control.

Yield and Yield Components

Grain yield was significantly influenced by herbicides-pyroligneous mixture through panicle formation and spikelet fertility (Table 4). All treatments gave higher number of panicles per hill than control. Treatments with AC 50%, AC 50% +RV500 and BCB 50% significantly had higher number of panicles over the control. Spikelet number per panicle, MPE 100%, MPE 50%, MPE 50% + WV500 and BCB 100% were higher compared to control while the AC treatments and BCB 50% and BCB 50%+RV500 had significantly lower number of spikelet than control. The ripened grain ratio and 1000 grain weight was similar between treatments including control. Higher grain yield was obtained in treatments combination involving MPE and AC except for AC 50%. In BCB treatments, only BCB 50% had significantly higher grain yield over the control. Higher grain yield in MPE treatments could be due in part to high spikelet number per panicle and in AC treatments due to higher panicle formation.

Grain Quality

Herbicides-pyroligneous acids mixtures affected grain quality in terms of protein content (Table 5), and milling quality and kernel chalkiness (Table 6). The TOYO taste value and starch amylose did not vary among treatments and lower protein content was observed in BCB50% +WV500 compared with the control. All other treatments had higher or comparable protein content than control (Table 5). All treatments involving MPE including WV and RV dilutions had higher whole grain yield, low total damage and heat damage grain compared to control (Table 6). In AC treatments, only the AC50% + WV500 had higher whole grain and lower damage grains over the control. All others treatment with AC herbicide had comparable amount of whole grain or higher damage grains compared to control. In BCB treatments, 100% BCB and BCB 50% + WV 500 had significantly higher whole grain yield and low heat damage grain compared with control. Rice grain chalkiness and amount of cracked grains were not significantly affected by different treatments.

Soil Chemical Properties and Nutrient Availability

Soil chemical properties with significant difference between treatments were soil pH, EC, organic matter (OM), available P, exchangeable Mg and total N while available silicon, exchangeable K and Ca did not vary significantly among treatments (Table 7). The soil pH changed to acidic after crop harvest compared to initial pH before planting (Table 7). Based on pH of control plots after crop harvest, there was no significant difference observed among treatments except that MPE 50% + RV500 and BCB 50% + RV 500 had considerably lower pH than control. Similarly, the EC decreased in all treatments after crop harvest and based on EC of control after harvest, there was no significant differences among treatments. Total organic matter and total N content increased after crop harvest compared to the initial levels before planting. Higher organic matter content was observed in AC 50%+RV500, MPE 50%+WV500 and MPE 50%+RV based on the initial organic matter levels before the experiment. The total N in soil increased after planting in herbicides and/or pyroligneous acid treatments especially in AC50%, AC50%+RV500 and BCB50%. The available P in all treatments was significantly higher than initial P levels in soil before planting but there was no significant difference observed between treatments after harvest. On other hand, the exchangeable Mg generally declined after harvest except on treatments AC50%+WV500 and MPE50%+RV500 where Mg levels were comparable to initial exchangeable Mg in soil before planting.

Discussion

Combined application of pyroligneous acids with lower rate of herbicides results in varying efficacies depending on the

Table 2: Effectiveness of herbicides-pyroligneous acids mixtures against annual and perennial weeds in paddy fields 30 days after treatment application[1]

Treatments	Annuals						Perennials		
	Echinochloa crusgalli			*Monochoria vaginalis*			*Scirpus juncoides*		
	Fresh weight (g)	Dry weight (g)	Efficacy (%)	Fresh weight (g)	Dry weight (g)	Efficacy (%)	Fresh weight (g)	Dry weight (g)	Efficacy (%)
Azimsulfuron+cafenstrole									
AC 100%	0.00 b	0.00 b	100.00	2. 70 b	0.37 b	95.57	0.00 b	0.00 b	100.00
AC 50%	0.00 b	0.00 b	100.00	6. 70 b	1.00 b	85.33	0.00 b	0.00 b	100.00
AC 50% + WV 500	0.00 b	0.00 b	100.00	1. 30 b	1.00 b	85.38	0.00 b	0.00 b	100.00
AC 50% + RV 500	0.00 b	0.00 b	100.00	5. 30 b	1.41 b	79.34	0.00 b	0.00 b	100.00
Mefenacet+pyrazosulfuron-ethyl									
MPE 100%	0.00 b	0.00 b	100.00	1. 30 b	0.25 b	96.30	0.00 b	0.00 b	100.00
MPE 50%	0.00 b	0.00 b	100.00	1. 30 b	0.16 b	97.67	0.00 b	0.00 b	100.00
MPE 50% + WV 500	0.00 b	0.00 b	100.00	5. 30 b	0.67 b	90.25	0.00 b	0.00 b	100.00
MPE 50% + RV 500	0.00 b	0.00 b	100.00	0.00 b	0.00 b	100.00	0.00 b	0.00 b	100.00
Bentazon+cyhalofop-butyl									
BCB 100%	0.00 b	0.00 b	100.00	1.30 b	0.99 b	85.58	1.33 a	0.17 a	38.09
BCB 50%	1.30 a	4.87 a	19.07	4.00 b	2.39 b	65.11	0.00 b	0.00 b	100.00
BCB 50% + WV 500	0.00 b	0.00 b	100.00	4.00 b	1.88 b	72.51	0.00 b	0.00 b	100.00
BCB 50% + RV 500	0.00 b	0.00 b	100.00	4.00 b	2.36 b	65.50	0.00 b	0.00 b	100.00
NT(control)	2.67 a	6.01 a		10.67 a	6.84 a		1.33 a	0.28 a	

[1]Same letters within the same parameters are not significantly different at P < 0.05 level by LSD

Table 3: Effects of different mixtures of organic materials and chemical fertilizer on plant height (PH), tiller number (TN), and photosynthesis at maximum tillering and heading stages of rice[1]

	Tillering Stage			Heading Stage		
Treatments	Height (cm)	Tiller Number	Chlorophyll Content (SPAD Value)	Height (cm)	Tiller Number	Chlorophyll Content (SPAD Value)
Azimsulfuron+cafenstrole						
AC 100%	52.13 a	34.20 a	30.50 ab	90.20 ab	19.90 a	27.75 ab
AC 50%	52.07 a	34.20 a	31.30 a	89.86 ab	19.40 a	31.10 a
AC 50% + WV 500	51.27 a	34.80 a	32.80 a	89.86 ab	19.30 a	32.05 a
AC 50% + RV 500	51.13 a	34.40 a	32.40 a	90.46 a	20.50 a	30.95 a
Mefenacet+pyrazosulfuron-ethyl						
MPE 100%	51.80 a	36.50 a	30.05 ab	90.13 ab	19.50 a	29.60 a
MPE 50%	51.47 a	34.00 a	33.20 a	90.33 ab	19.50 a	26.70 ab
MPE 50% + WV 500	52.20 a	34.60 a	32.20 a	90.33 ab	19.50 a	29.55 a
MPE 50% + RV 500	52.00 a	35.20 a	30.15 ab	90.60 a	20.90 a	28.10 a
Bentazon+cyhalofop-butyl						
BCB 100%	51.87 a	34.20 a	29.20 b	90.07 ab	19.30 a	31.55 a
BCB 50%	51.67 a	34.90 a	28.00 b	89.60 b	20.00 a	30.05 a
BCB 50% + WV 500	51.73 a	34.70 a	31.80 a	89.87 ab	19.70 a	29.05 a
BCB 50% + RV 500	51.67 a	34.20 a	28.90 b	89.80 ab	19.10 a	29.85 a
NT(control)	52.20 a	35.50 a	28.00 b	89.37 b	18.80 a	25.75 b

[1]Same letters within the same parameters are not significantly different at P < 0.05 level by LSD

Table 4: Effects of different mixtures of herbicides and pyroligneous acids on the yield components of rice[1]

Treatments	Panicle/Hill	Spikelet/Panicle	Ripened Grain Ratio	1,000 Grain Weight (g)	Yield (kg/ha)
Azimsulfuron+cafenstrole					
AC 100%	18.73 ab	73.50 c	82.97 a	23.87 a	6440.80 a
AC 50%	19.06 a	75.47 c	81.63 a	22.93 a	6095.70 ab
AC 50% + WV 500	18.86 ab	79.23 b	81.97 a	23.87 a	6344.50 a
AC 50% + RV 500	19.00 a	73.63 c	85.33 a	24.07 a	6277.90 a
Mefenacet+pyrazosulfuron-ethyl					
MPE 100%	18.47 ab	87.60 a	84.27 a	24.43 a	6320.80 a
MPE 50%	18.13 ab	83.40 a	81.67 a	24.00 a	6260.15 a
MPE 50% + WV 500	17.93 b	85.23 a	84.50 a	22.87 a	6350.50 a
MPE 50% + RV 500	18.80 ab	78.90 b	85.53 a	24.90 a	6304.60 a
Bentazon+cyhalofop-butyl					
BCB 100%	18.73 ab	83.76 a	83.50 a	23.80 a	6160.90 ab
BCB 50%	19.13 a	66.16 c	84.90 a	23.90 a	6283.80 a
BCB 50% + WV 500	18.00 b	76.23 b	81.43 a	25.23 a	6165.30 ab
BCB 50% + RV 500	18.73 ab	68.26 c	84.13 a	24.60 a	6048.30 ab
NT(control)	17.60 b	79.56 b	85.37 a	24.03 a	5722.40 b

[1]Same letters within the same parameters and herbicide type are not significantly different at P < 0.05 level by LSD

type of herbicides and target weed species (Cueto, 2012). In this experiment, we found promising combination of herbicides-pyroligneous acids under field conditions and assessed its effect on rice grain quality and potential impacts on soil properties. Combined application of pyroligneous acids with herbicides could directly influence the herbicidal activity or indirectly alter plant- and soil-herbicide interactions. The herbicides-pyroligneous acids mixtures effectively controlled *E. crusgalli* and *S. juncoides*; and combinations of 500 × dilution with RV or WV enhanced the efficacy of these herbicides in controlling *M. vaginalis* comparable to 100% herbicide application. These results conformed to herbicidal efficacy obtained from mixing RV and WV with these particular herbicides to control paddy weeds (Cueto, 2012; Acenas *et al.*, 2013). Although, we cannot fully account the herbicides by pyroligneous acid interaction which enhanced herbicidal activity and this increase could be due to the effects of acidity and nutrient contents of RV and WV. Depending on the chemical properties of both the pyroligneous acid and herbicides, combined application of pyroligneous acids could either increase or decrease the herbicide solubility due to its acidity and hence absorption and efficacy. The acidity of RV and WV could also react with herbicide active ingredient that could either increase or decrease its herbicidal activity (Liu, 2002). For instance, the sorption of mefenacet on soil and its components generally decreased with increasing pH and mefenacet was very stable and did not hydrolyze in pH range of 3−10 in 5 days (Huang *et al.*, 2002). Zheng *et al.* (2008) indicated that degradation of herbicide is pH-dependent and herbicide was least persistent in acidic pH followed by alkaline and neutral pH. The half-life of pyrazosulfuron-ethyl varied from 2.6 (pH 4) to 19.4 days (pH 7) and half-life in distilled water was comparable to half-life at pH 7 buffer (Pal *et al.*, 2006; Zheng *et al.*, 2008). The mineral and organic components of RV and WV could influence the mobility, availability and resilience of herbicide in soil thus affecting its overall efficacy. Blasioli *et al.* (2008) showed that presence of dissolved organic matter did not modify the degradation pattern but only reduced the adsorption of cyhalofop-butyl in soil, whereas it increased the adsorption of cyhalofop-acid. Furthermore, the acidity and nutrient components of pyroligneous acids could modify the soil microenvironment by altering soil pH and providing source of substrates for microbial activity (Kadota and Niimi, 2004; Steiner *et al.*, 2008). Depending on the property of herbicides, acidification could either increase or decrease the solubility and mobility in soil, and availability for plant absorption. It could also enhance microbial degradation of herbicide in soil hence lowering its residence time and effectiveness. The specific interaction of RV or WV with different herbicides needs to be pursued in future studies.

The nutrients, enzymes and other organic components of pyroligneous acids could either enhance or decrease plants ability to metabolize the herbicide, which could result to varying responses including plant's susceptibility or resistance to herbicide, plant nutrition and growth, and overall quality of rice grains (Steiner *et al*., 2008; Zulkarami *et al*., 2011). The growth performance of rice plants treated with 50% RR herbicides + pyrolignous acid mixtures were superior to control and comparable if not better than 100% herbicide treatment. This indicate that aside from improved herbicidal efficacy of 50% RR herbicides+pyroligneous acid combination against paddy weeds, it could also enhanced plant growth as indicated by taller plants especially with combinations of AC100+RV500 and MPE50%+RV500. The chlorophyll content was also improved by RV and WV especially in combination with AC and MPE herbicides. The improvement of plant growth in treatments with RV and WV could be due to additional nutrients and organic substances present in pyroligneous vinegar are beneficial for plants. In addition, the chlorophyll content of rice receiving RV and WV in combination with 50% RR of herbicides was relatively higher compared to full rate of herbicide application indicating reduced toxicity of herbicides on rice plant (Thapa, 2013). Although there were no significant differences between treatments, the productive tiller count was improved with RV application especially in combination with AC50% and MPE50%. Several studies have shown the positive effects of pyroligneous acid on plant growth and such benefits retained when combined with herbicides (Jianming, 2003; Pangnakorn *et al*., 2009; Altland and Locke, 2013; Masum *et al*., 2013). Improvement of growth could be attributed to the effect of nutrient contents of RV and WV that aids in the rice plant nutrition.

Table 5: Effects of different mixtures of herbicides and pyroligneous acids on palatability, protein and amylase contents[1]

Treatments	TOYO value	Protein	Starch Amylose (%)
Azimsulfuron+cafenstrole			
AC 100%	81.50 a	6.43 a	19.33 a
AC 50%	80.60 a	6.47 a	19.50 a
AC 50% + WV 500	81.60 a	6.37 a	20.23 a
AC 50% + RV 500	81.03 a	6.27 ab	20.00 a
Mefenacet+pyrazosulfuron-ethyl			
MPE 100%	83.67 a	6.20 ab	20.10 a
MPE 50%	84.00 a	6.13 ab	19.97 a
MPE 50% + WV 500	84.77 a	6.23 ab	20.00 a
MPE 50% + RV 500	84.23 a	6.30 a	19.80 a
Bentazon+cyhalofop-butyl			
BCB 100%	83.00 a	6.27 ab	20.17 a
BCB 50%	81.40 a	6.46 a	19.80 a
BCB 50% + WV 500	84.30 a	6.06 b	19.63 a
BCB 50% + RV 500	81.53 a	6.17 ab	19.53 a
NT(control)	83.73 a	6.20 ab	19.57 a

[1]Same letters within the same parameters and herbicide type are not significantly different at $P < 0.05$ level by LSD

Rice quality is affected by environmental conditions and cultural practices during growth aside from genetic control. Combined application of RV and WV with herbicides did not compromise the palatability and

Table 6: Effects of different mixtures of herbicides and pyroligneous acids on grain quality[1]

Treatments	Whole	Broken	Chalky	Damaged	Heat-Damaged	Cracked
Azimsulfuron+cafenstrole						
AC 100%	77.50 b	5.87 b	1.90 a	14.13 a	0.63 a	15.93 a
AC 50%	81.50 b	7.10 b	1.33 a	9.63 a	0.40 ab	15.23 a
AC 50% + WV 500	86.50 a	10.47 a	1.73 a	1.20 c	0.00 c	17.13 a
AC 50% + RV 500	84.70 ab	7.23 ab	2.43 a	5.20 a	0.40 ab	17.70 a
Mefenacet+pyrazosulfuron-ethyl						
MPE 100%	87.50 a	9.73 ab	1.50 a	1.27 c	0.07 c	17.43 a
MPE 50%	87.40 a	9.53 ab	1.33 a	1.70 c	0.07 c	21.73 a
MPE 50% + WV 500	86.20 a	10.63 a	1.50 a	1.57 c	0.03 c	17.97 a
MPE 50% + RV 500	88.70 a	7.87 ab	1.93 a	1.43 c	0.03 c	18.57 a
Bentazon+cyhalofop-butyl						
BCB 100%	87.20 a	8.97 ab	1.70 a	2.10 c	0.07 c	17.57 a
BCB 50%	82.30 b	8.77 ab	2.47 a	6.00 b	0.47 a	16.40 a
BCB 50% + WV 500	85.40 a	10.67 a	1.33 a	3.13 c	0.17 bc	19.33 a
BCB 50% + RV 500	80.40 b	10.57 a	1.43 a	7.27 ab	0.30 b	19.07 a
NT(control)	82.10 b	11.10 a	1.33 a	5.27 a	0.17 bc	16.30 a

[1]Same letters within the same parameters are not significantly different at $P < 0.05$ level by LSD

Table 7: Soil chemical properties of paddy soil as affected by herbicide + pyroligneous mixture application[1]

Treatments	pH	SiO_2	OM	P_2O_5	K	Ca	Mg	EC	T-N
		(mg kg^{-1})	(g kg^{-1})	(mg kg^{-1})		(cmol$^+$ kg^{-1})		(dS m^{-1})	
Azimsulfuron+cafenstrole									
AC 100%	5.40 ab	63.67 a	28.37 ab	121.70 a	0.21 a	3.71 a	0.93 b	0.30 ab	0.24 ab
AC 50%	5.26 b	61.33 a	28.36 ab	129.70 a	0.19 a	3.56 a	0.99 b	0.30 ab	0.25 a
AC 50% + WV 500	5.50 ab	67.00 a	28.34 ab	125.70 a	0.22 a	3.84 a	1.47 a	0.38 ab	0.24 ab
AC 50% + RV 500	5.56 a	68.67 a	30.66 a	133.00 a	0.21 a	3.84 a	1.21 ab	0.27 b	0.25 a
Mefenacet+pyrazosulfuron-ethyl									
MPE 100%	5.40 ab	59.67 a	28.18 ab	122.00 a	0.23 a	4.06 a	1.08 b	0.31 ab	0.24 ab
MPE 50%	5.53 ab	68.67 a	27.51 ab	129.00 a	0.19 a	3.96 a	0.91 b	0.23 b	0.24 ab
MPE 50% + WV 500	5.33 ab	65.00 a	29.86 a	140.00 a	0.24 a	3.79 a	1.27 ab	0.36 ab	0.23 ab
MPE 50% + RV 500	5.27 b	68.00 a	29.26 a	136.00 a	0.21 a	3.52 a	1.38 a	0.29 ab	0.23 ab
Bentazon+cyhalofop-butyl									
BCB 100%	5.33 ab	67.67 a	28.79 ab	127.70 a	0.23 a	3.63 a	1.26 ab	0.28 b	0.24 ab
BCB 50%	5.33 ab	63.00 a	28.38 ab	121.70 a	0.20 a	3.76 a	1.06 b	0.26 b	0.26 a
BCB 50% + WV 500	5.40 ab	83.00 a	28.25 ab	116.00 ab	0.19 a	3.68 a	1.19 b	0.27 b	0.23 ab
BCB 50% + RV 500	5.17 b	65.00 a	27.76 ab	128.70 a	0.19 a	3.29 a	0.95 b	0.25 b	0.23 ab
NT(control)	5.37 ab	65.00 a	28.32 ab	119.30 a	0.21 a	3.62 a	1.05 b	0.27 b	0.22 b
NTB(control-before planting)	5.50 ab	65.67 a	24.97 b	72.30 b	0.24 a	3.83 a	1.37 a	0.47 a	0.22 b

[1]Same letters within the same parameters are not significantly different at $P < 0.05$ level by LSD

nutritional content of rice as indicated by the TOYO taste values and the starch amylose and protein contents. Slight reduction in protein content was observed in treatments involving RV and WV with 50% RR of MPE and BCB which resulted to slight improvement of palatability. It is established that high protein content in grains is negatively correlated to TOYO-taste values or palatability (Choi and Choi, 2006). However, our data only shows minimal variation in protein and amylose contents that only resulted to slight differences of palatability (by TOYO-taste values). Although previous studies have demonstrated how the pyroligneous acid-herbicide mixture altered the protein synthesis and contents on target weed species (Acenas *et al*., 2013), the mechanisms by which these treatments have altered the protein contents in rice grains warrants future investigations.

Our results showed that effect of RV and WV on grain quality depends on the type of herbicides and rate of application. The improvement of grain quality observed in AC treatment could be attributed to effect of RV and WV on plant nutrition and plants' ability to recover against herbicide stress (Nowak and Shulaev, 2003; Kang *et al*., 2012; Lashari *et al.*, 2013). RV and WV contain essential nutrients that favor this overall positive performance of yield quality (Zulkarami *et al.*, 2011; Kang *et al*., 2012).

The residual nutrients content in soil improved due to combined application of RV and WV with 50% herbicide rates as indicated by higher exchangeable K, Ca, Mg, available SiO_2 and P_2O_5 and total OM and nitrogen. This is an indication of the nutrient supplying potential of pyroligneous acids when applied to soil which could be beneficial to plant nutrition even at reduce dosage. Pyroligneous acid promote nutrient availability such as N utilization efficiency and P availability; and improve the overall soil health by inducing microbial activities and functions (Steiner *et al*., 2008; Chun-hua *et al*., 2012). We supposed that the same effect occurred from the application of RV and WV in combination with reduced amount of herbicides on soil nutrient dynamics and microbial activities in this study.

Conclusion

Mixing 50% RR of herbicides with rice vinegar or wood vinegar at 500× can effectively control the weeds in paddy rice such as *E. crus-galli, M. vaginalis* and *S. juncoides* without suppressing the growth, and yield of rice. It also increased the soil acidity, organic matter, available P and EC, which resulted in higher residual nutrients in soil. Utilization of herbicide-pyroligneous acid mixtures improved nutrient availability and can be a major contributing factor in minimizing the use of synthetic chemicals in crop production for protection of environment and human health.

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