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GROWTH EFFECT OF THE DIFFERENT RATIOS OF WOOD VINEGAR ON *Brassica juncea*

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ABSTRACT

Four (4) different ratios of wood vinegar namely; 1:5; 1:10; 1:20 and 1:30 mL WV to water ratio extracted from coconut shell was tested to determine their growth effect on *Brassica juncea* or mustard plant or locally known as mustasa. Parameters measured were leaf number, plant height, plant leaf width. Using randomized complete block design, experimental tests were conducted using *Brassica juncea* as test plants. The samples of 10 days old were allowed to flourish in a potted plant with day to day application of the wood vinegar solutions together with the negative control for a total of 30 days. Results revealed that the wood vinegar ratios applied have no significant effect on the growth of the mustard plant in terms of its leaf number, plant height and leaf width. This indicates that coconut shell wood vinegar at 1:5, 1:10, 1:20 and 1:30 are not applicable ratios for plant production, as that an increased wood vinegar application increases plant production and growth.

Keywords: Wood Vinegar, *Brassica juncea*, Organic Fertilizer, Wood Vinegar Ratio

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INTRODUCTION

As commonly known, chemical fertilizers which are usually utilized in the agricultural industry can cause the soil being sprayed to be too acidic and leads to another problem in the environment; water pollution. Wood vinegar is the substance produced through the condensation of smoke emitted during the pyrolysis of wood and its residues from processing. It is an essential substance that promotes healthy method for propagating plants, and also, it can be used as fertilizer or soil conditioner. Several researches have already evidenced the great impact of using wood vinegar for elevating the nutrient level of soil.

According to Payamara, J. (2011), the major component of wood vinegar products are acetic acid, methanol, propanoic acid, phenolic and carbonyl compounds. The wood vinegar improves soil quantity eliminates pests, accelerating plant growth, plant growth regulator or growth inhabiting. The bio–test of wood vinegar inhibits the growth of Xanthomonas comprestris pv. The wood vinegar was applied on maize with spraying on leaf compare with spraying on soils every 6 days after planting. The acidity range 1.95 to 2.14 the major component in wood vinegar was observed to be acetic acid. According to Thailand's Department of Agriculture (2010), wood vinegar can improvement of soil quality, eliminates of plant and soil pests, controls plant growth, is able to accelerate the development of roots, stems, tubers, leaves, flowers, and fruits, and, increases amounts of fruit produced in orchards. According to Brunette, R. (2010), wood vinegar is produced when smoke from charcoal production is cooled by outside air while passing through a chimney or flue pipe. The cooling effect causes condensation of pyroligneous liquor, particularly when the temperature of smoke produced by carbonization ranges between 80 and 180ºC/176 and 356ºF (Nikhom, 2010). This temperature is reached at the carbonization stage of exothermic decomposition (see previous article about charcoal production) and is indicated by the production of yellowish, acrid smoke. Moreover, Tancho, A. reported that wood vinegar can be applied to the soil surface to help increase the population of beneficial microbes and to promote plant root growth. Additionally, the product can help boost crop defenses against disease. A strong solution of wood vinegar with a 1:30 ratio application to the garden soil surface at a rate of 6 liters of solution per 1m² will enrich the soil prior to planting crops. Also, it can be used to control soil-based plant pathogens with an even stronger rate of 1:5 to 1:10 ratio. This prompted the researchers to further add literature and studies to the growing interest of the scientific community in utilizing the many uses of wood vinegar in the agricultural sector, to further prove the best exploit of wood vinegar in backyard soil to further determine if the application of wood vinegar for agriculture is reasonable. This study

anchors on determining the effects of different ratio proportion of wood vinegar sourced out from coconut shell on the physical aspects of *Brassica juncea* (Mustard plant). More specifically, this study aims to determine the growth effect of the different coconut shell ratio on the growth of Mustard plant in terms of (1) number of leaves, (2) plant height, (3) plant leaf width. Further to determine which ratio of wood vinegar (Wood Vinegar to Water) is more suitable for growing mustard plants in the following ratio levels; 1:5, 1:10, 1:20, and 1:30. Lastly, to determine if there is a significant difference on the growth effect of the different wood vinegar ratio on mustard plant.

METHODOLOGY

Research Design

This study will employ the Experimental Research Design in which Randomized Complete Block Design (RCBD) will be used for determining the growth effect of the different ratios of wood vinegar extracted from coconut shell.

Instrumentation and Data Gathering procedure

Dry distillation of coconut shell will be done using procedures from Phywe (2017). The dry distillation (as seen on Figure 1) was assembled by the researchers for a faster rate of extracting wood vinegar. Experimental procedures were done under laboratory conditions, all in triplication to minimize errors. Extra care was also utilized in this experiment for explosive and toxic fumes are emitted during distillation.

Figure 1. Kiln for Extracting Wood Vinegar

Instruments of the Study

In the following figure (Figure 2 and 3) the instruments and reagents that were used in the study are specified:

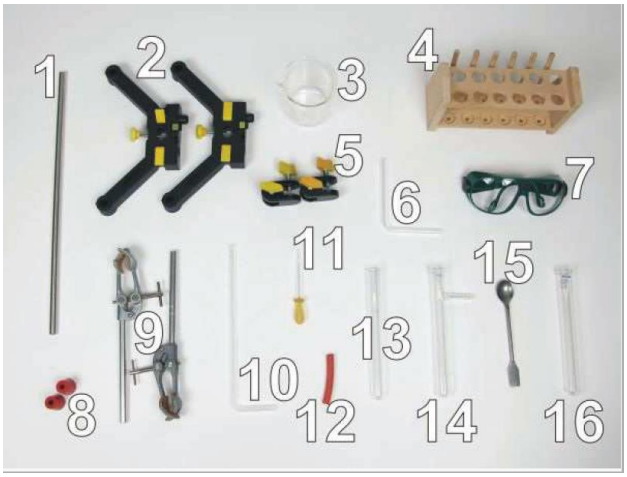

Figure 2. Equipment used in Dry distillation

Wood Vinegar Ratio

The wood vinegar produced from the dry distillation of coconut shell will be diluted in the manner of ratio (Table 1):

Table 1. Wood Vinegar to Water Ratio

Ratio	Amount of WV	Amount of H_2O	Total volume
1:5	10 mL	50 mL	60 mL
1:10	10 mL	100 mL	110 mL
1:20	10 mL	200 mL	210 mL
1:30	10 mL	300 mL	310 mL

The following ratio are adopted following the suggestions made by Tancho, A. (n.d.) wherein a strong solution of wood vinegar with a 1:30 ratio application to the garden soil surface at a rate of 6 liters of solution per $1m^2$ will enrich the soil prior to planting crops. Also, it can be used to control soil-based plant pathogens with an even stronger rate of 1:5 to 1:10 ratio.

Growth Effect of Wood Vinegar to *Brassica juncea*

Planting and Propagating Mustard Samples

Ten (10) days old mustard plants will be utilized in this study. The mustard plants will be potted and divided into 5 groups. The ratio groups consisted of 1:5, 1:10, 1:20 and 1:30 ratios and the control group which is the negative control (no wood vinegar added).

The division of groups will follow the randomized complete block design (RCBD) which is as follows which will have three trials and three replications (Table 2):

Table 2. Randomized Complete Block Design

1:5 ratio			1:10 ratio			1:20 ratio			1:30 ratio			N. control		
T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1
T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2

The potted plant samples will be put in open area and will be watered everyday twice daily using tap water. The plant samples will be allowed to flourish to its maturity for 30 to 35 days while continuous adding of 0.5 mL wood vinegar ratio is done once daily. After the maturity date, the plants will be harvested in the morning and will be subjected to the following parameters; a) Number of leaves, b) plant height, and c) Plant leaf width.

Number of Leaves

The number of leaves of the samples will be counted manually and will be recorded. Average number per trial will be computed based.

Plant Height

Plant height will be determined manually using a tape measure and then recorded. Average height per trial will also be computed. The computation will be from the base at which the plant and the soil is touching and up to the topmost part of the plant.

Plant Leaf Width

Plant leaf width will be determined manually using a tape measure and then recorded. The widest plant leaf in the sample will be used as representative of the plant for this kind of test. Average width per trial will also be computed.

Statistical Analysis of Data

One-way Analysis of Variance (ANOVA) was used in this study to determine if there is a significant difference on the growth effect of the different wood vinegar ratio on mustard plants using IBM SPSS version 28.

RESULTS AND DISCUSSION

The following data were gathered after thorough experimentation and analysis:

Growth Effects

A number of plant parameters were measured to determine the growth effect of varying wood vinegar ratios on *Brassica juncea*. The following plant factors were taken into consideration:

Number of leaves

Leaf number was counted before application and 30 days after the propagation of the plant to determine if the application of wood vinegar would increase or decrease the total number of leaves that can be propagated by the plant species. The following results were obtained (Table 3):

Table 3. Results on leaf number before and after administering different wood vinegar ratios

WV Ratio	Replicates	Day 1			Day 30		
		T_1	T_2	T_3	T_1	T_2	T_3
1:5	1	5	5	7	9	5	6
	2	6	6	5	7	8	7
	3	5	5	6	7	8	6
	Average	***5.33***	***5.33***	***6***	***7.67***	***7***	***6.33***
1:10	1	5	6	6	6	7	6
	2	6	7	6	5	9	7
	3	7	6	7	8	8	7
	Average	***6***	***6.33***	***6.33***	***6.33***	***8***	***6.67***
1:20	1	5	5	6	5	6	6
	2	5	5	6	5	7	6
	3	6	7	6	7	7	5
	Average	***5.33***	***5.67***	***6***	***5.67***	***6.67***	***5.67***
1:30	1	7	6	6	8	8	5
	2	6	6	7	6	6	6
	3	5	6	7	6	6	4
	Average	***6***	***6***	***6.67***	***6.67***	***6.67***	***5***
Untreated	1	8	7	5	6	6	6

(Negative Control)	**2**	6	6	6	7	6	7
	3	4	7	7	6	7	6
	Average	***6***	***6.67***	***6***	***6.33***	***6.33***	***6.33***

The results above were gathered during day 0 and day 30 of the entire study after administering 0.5 mL of different ratios of wood vinegar and water together with the negative control. It can be observed that a slight change in the number of leaves was observed on all ratios while almost no change was observed on the untreated group.

Plant Height

Leaf height was measured to determine if plants samples administered to different levels of wood vinegar would grow taller or not. These were the results obtained at the millimeter (mm) unit (Table 4):

Table 4. Results on plant height before and after administering different wood vinegar ratios

WV Ratio	Replicates	Day 1 (mm)			Day 30 (mm)		
		T_1	T_2	T_3	T_1	T_2	T_3
1:5	**1**	70	42	44	225	163	113
	2	50	61	49	201	215	208
	3	61	46	55	171	210	132
	Average	***60.33***	***49.67***	***49.33***	***199.0***	***196.0***	***151.0***
1:10	**1**	67	70	70	135	144	134
	2	65	60	75	150	223	141
	3	49	74	68	173	211	213
	Average	***60.33***	***68.0***	***71.0***	***152.67***	***192.67***	***162.67***
1:20	**1**	72	49	51	162	138	139
	2	52	64	74	131	162	181
	3	102	100	81	216	232	140
	Average	***75.33***	***71.0***	***68.67***	***169.67***	***177.33***	***153.33***
1:30	**1**	76	59	68	222	171	159
	2	55	41	74	128	133	151
	3	78	54	62	164	190	121
	Average	***69.67***	***51.33***	***68.0***	***171.33***	***164.67***	***143.67***
Untreated (Negative Control)	**1**	64	50	68	147	156	162
	2	74	52	89	160	155	162
	3	39	78	76	168	161	181
	Average	***59.0***	***60.0***	***77.67***	***158.33***	***157.33***	***168.33***

The results above were gathered during day 0 and day 30 of the entire study after administering 0.5 mL of different ratios of wood vinegar and water together with the negative control. It can be observed that a great change in plant height was observed on all ratios as well as on the untreated group.

Plant Leaf Width

Leaf width was measured to determine if plants samples administered to different levels of wood vinegar will have green broader leaves which indicates healthy and well-rounded

growth of the plant samples. These were the results obtained at the millimeter (mm) unit (Table 5):

Table 5. Results on leaf width before and after administering different wood vinegar ratios

WV Ratio	Replicates	Day 1 (mm)			Day 30 (mm)		
		T_1	T_2	T_3	T_1	T_2	T_3
1:5	1	32	18	31	96	67	48
	2	26	36	27	71	82	75
	3	27	22	26	69	75	54
	Average	***28.33***	***25.33***	***28.0***	***78.67***	***74.67***	***59.0***
1:10	1	42	34	38	51	55	56
	2	34	27	29	46	92	55
	3	26	35	31	57	56	80
	Average	***34.0***	***32.0***	***32.67***	***51.33***	***67.67***	***63.67***
1:20	1	30	41	30	59	65	54
	2	22	34	32	57	61	57
	3	38	33	34	79	68	54
	Average	***30.0***	***36.0***	***32.0***	***65.0***	***64.67***	***55.0***
1:30	1	31	22	32	71	80	55
	2	32	18	38	50	55	66
	3	33	25	34	55	70	30
	Average	***32.0***	***21.67***	***34.67***	***58.67***	***68.33***	***50.33***
Untreated (Negative Control)	1	28	33	25	66	55	49
	2	31	21	48	74	55	56
	3	24	36	34	60	65	67
	Average	***27.67***	***30.0***	***35.67***	***66.67***	***58.33***	***57.33***

From day 0 to day 30, the results gathered during the entire study after administering 0.5 mL of different ratios of wood vinegar and water together with the negative control showed a change in the plant leaf width which was observed on all ratios as well as on the untreated group.

Statistical Analysis

The ANOVA table 6 was generated using IBM SPSS version 28 (free trial) indicating that there are no significant differences between the wood vinegar ratios with that of the negative control (factor control). This indicates result eliminates the bias on the start of the conduct of research (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021). Also, the table above showed no significant difference between individual wood vinegar ratios and the untreated group or control group since F computed values are all below the T-computed values indicated by the table above.

Table 6. ANOVA Table for Pre-treatment (Leaf Number)

		Sum of Squares	df	Mean Square	F	Sig.
1:5 ratio (Leaf	Between Groups	.075	1	.075	.333	.667

Number) Day 1	Within Groups	.224	1	.224		
	Total	.299	2			
1:10 ratio (Leaf Number) Day 1	Between Groups	.018	1	.018	.333	.667
	Within Groups	.054	1	.054		
	Total	.073	2			
1:20 ratio (Leaf Number) Day 1	Between Groups	.000	1	.000	.000	.995
	Within Groups	.224	1	.224		
	Total	.224	2			
1:30 ratio (Leaf Number) Day 1	Between Groups	.075	1	.075	.333	.667
	Within Groups	.224	1	.224		
	Total	.299	2			

However, after 30 days of application of the different wood vinegar ratios together with the negative control showed minimal difference indicated by the table 7 below:

Table 7. Warnings on ANOVA Post-treatment (Leaf Number)

There are fewer than two groups for dependent variable 1:5 ratio (Leaf Number) Day 30. No statistics are computed.
There are fewer than two groups for dependent variable 1:10 ratio (Leaf Number) Day 30. No statistics are computed.
There are fewer than two groups for dependent variable 1:20 ratio (Leaf Number) Day 30. No statistics are computed.
There are fewer than two groups for dependent variable 1:30 ratio (Leaf Number) Day 30. No statistics are computed.

Now since the data has fewer groups, the researchers run MVA or Missing Value Analysis to determine where the data sets are missing, the results are shown in the table 8 below:

Table 8. Univariate Statistics (Leaf Number)

	N	Mean	Std. Deviation	Missing		No. of Extremes[a]	
				Count	Percent	Low	High
day1.1.5.ln	3	5.5533	.38682	11	78.6	0	0
day1.1.10ln	3	6.2200	.19053	11	78.6	0	0
day1.1.20ln	3	5.6667	.33501	11	78.6	0	0
day1.1.30ln	3	6.2233	.38682	11	78.6	0	0
day1.NCln	3			11	78.6		

a. Number of cases outside the range (Q1 - 1.5*IQR, Q3 + 1.5*IQR).

As seen from the table above, since the number of extremes in both high and low is zero (0) this indicates that in all the ratios together with the negative control indicate no difference between them since univariate statistics show 78.6% missing values and an almost similar standard deviation between samples. It means that all individual samples from different groups have almost the same leaf number count or result thus there is no variability in that leaf number parameter. This result indicates that there are no significant differences between individual ratios versus the negative control and that, after 30 days of administering wood

vinegars, in terms of leaf number, the wood vinegar ratios and the untreated groups have similar result (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021).

This result varies with the result conducted by Zhu et al. (2021) wherein they stated that different treatments of wood vinegar tended to increase the total leaf number and green leaf number of their samples which are rapeseed in the pod stage. However, their result also suggested that the combination of wood vinegar and melatonin most effectively increased the total leaf number. It was found out based on ANOVA that the difference was insignificant in all treatments over the control plots.

Table 9. ANOVA Pre-treatment (Plant Height)

		Sum of Squares	df	Mean Square	F	Sig.
1:5 ratio (Plant Height) Day 1	Between Groups	78.250	2	39.125	.	.
	Within Groups	.000	0	.		
	Total	78.250	2			
1:10 ratio (Plant Height) Day 1	Between Groups	60.559	2	30.280	.	.
	Within Groups	.000	0	.		
	Total	60.559	2			
1:20 ratio (Plant Height) Day 1	Between Groups	22.844	2	11.422	.	.
	Within Groups	.000	0	.		
	Total	22.844	2			
1:30 ratio (Plant Height) Day 1	Between Groups	205.678	2	102.839	.	.
	Within Groups	.000	0	.		
	Total	205.678	2			

The ANOVA table 9 above was generated using IBM SPSS version 28 (free trial) indicating that there are sum of squares within groups between the wood vinegar ratios with that of the negative control (factor control). This indicates result eliminates the bias on the start of the conduct of research. Also, the table above showed no difference between individual wood vinegar ratios and the untreated group or control group since F computed and T-computed values are not available due to 0 WSS (within sum f squares). This result also indicates that the data is a good model since value of zero in the sum of squares means the model used in the research is a perfect fit (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021).

ANOVA test in the post-treatment (Table 10) also revealed no significant difference between individual ratios and the negative control. As postulated above, the F and T values are not computed due to 0 results in the sum of squares. It was found out based on ANOVA above that the difference was insignificant in all treatments over the control plots.

Table 10. ANOVA Post-treatment (Plant Height)

		Sum of Squares	df	Mean Square	F	Sig.
1:5 ratio (Plant Height) Day 30	Between Groups	1446.000	2	723.000	.	.
	Within Groups	.000	0	.		
	Total	1446.000	2			
1:10 ratio (Plant Height) Day 30	Between Groups	866.667	2	433.333	.	.
	Within Groups	.000	0	.		
	Total	866.667	2			
1:20 ratio (Plant Height) Day 30	Between Groups	300.557	2	150.279	.	.
	Within Groups	.000	0	.		
	Total	300.557	2			
1:30 ratio (Plant Height) Day 30	Between Groups	416.810	2	208.405	.	.
	Within Groups	.000	0	.		
	Total	416.810	2			

Univariate statistics (Table 11) of the plant height result also revealed a 0 number on the low and high number of extremes which indicates that all individual samples from different groups have almost the same means or average, in terms of plant height or result thus there is no variability in that plant height parameter (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021).

Table 11. Univariate Statistics

	N	Mean	Std. Deviation	Missing		No. of Extremes[a]	
				Count	Percent	Low	High
day1.1.5.ln	3	5.5533	.38682	11	78.6	0	0
day1.1.10ln	3	6.2200	.19053	11	78.6	0	0
day1.1.20ln	3	5.6667	.33501	11	78.6	0	0
day1.1.30ln	3	6.2233	.38682	11	78.6	0	0
day1.NCln	3			11	78.6		

a. Number of cases outside the range (Q1 - 1.5*IQR, Q3 + 1.5*IQR).

This result is in contrast to the result conducted by Xin et al. (2017) wherein they stated that the wood vinegar alone addition significantly increased the plant height, root length, root volume and root tips of cucumber by 20.2%, 45.2%, 7.8% and 30.9%, respectively, compared to that of the CK treatment. However, based on the analysis of variance conducted by Travero & Mihara (2016) on their soybean research, it was found that the difference was insignificant in all treatments over the control plots in terms of their samples plant height which is in support to this study. The ANOVA table 12 was generated using IBM SPSS version 28 indicating that there are sum of squares within groups between the wood vinegar ratios with that of the negative control (factor control) in terms of plant leaf width also generated the same result as with the leaf height eliminating the bias on the start of the conduct of research. Also, the table above showed no difference between individual wood vinegar ratios and the untreated group or control group since F computed and T-computed values are not available due to 0 WSS (within sum of squares). This result also indicates that

the data is a good model since value of zero in the sum of squares means the model used in the research is a perfect fit (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021).

Table 12. ANOVA Pre-treatment (Leaf Width)

		Sum of Squares	df	Mean Square	F	Sig.
1:5 ratio (Leaf Width) Day 1	Between Groups	5.413	2	2.706	.	.
	Within Groups	.000	0	.		
	Total	5.413	2			
1:10 ratio (Leaf Width) Day 1	Between Groups	2.073	2	1.036	.	.
	Within Groups	.000	0	.		
	Total	2.073	2			
1:20 ratio (Leaf Width) Day 1	Between Groups	18.667	2	9.333	.	.
	Within Groups	.000	0	.		
	Total	18.667	2			
1:30 ratio (Leaf Width) Day 1	Between Groups	94.279	2	47.140	.	.
	Within Groups	.000	0	.		
	Total	94.279	2			

Similar results were also obtained during ANOVA test run in the post-treatment also revealed no significant difference between individual ratios and the negative control in terms of the plant height of the samples. As postulated above, the F and T values are not computed due to 0 results in the sum of squares (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021).

All data sets and results here were computed using IBM SPSS version 28 with emphasis on On-way Analysis of Variance (ANOVA), Missing Value Analysis (MVA), Post-hoc's were done using Tukey's and descriptive analysis in relation to mean plots (Aerd Statistics, 2018; EZ SPSS Tutorials, 2021). It was also found out based on ANOVA that the difference was insignificant in all treatments over the control plots. And that, this data is supported by the study of Travero & Mihara (2016), that application of wood vinegar has no influence on the growth of plants based on weekly recorded height of plants. Moreover, plants treated with 10% and 20% wood vinegar showed no significant difference as to its effect on yield.

CONCLUSIONS

The wood vinegar extracted from coconut shell at 1:5, 1:10, 1:20 and 1:30 are not suitable ratios for plant growth use. The wood vinegar extracted from coconut shell at 1:5, 1:10, 1:20 and 1:30 does not affect the growth of *Brassica juncea* in terms of the plant samples leaf number, plant height and leaf width. There are no significant differences between the leaf number, plant height and width of leaves based on the results on the statistical data analysis using 1-way Analysis of Variance (ANOVA). This study provides several recommendations such as; use an increased concentration of wood vinegar on the application to further

determine its effect, utilize other types of plant samples in determining efficacy of using wood vinegar in plant growth and as fertilizer, conduct a similar research to further oppose or affirm the results of this study, and conduct wood vinegar extraction on other wood materials.

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