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Reducing the Use of Diuron-Active Herbicides Using a Combination of Wood Vinegar from Lignocellulosic Waste in Pineapple Plantations

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Abstract

Pineapple waste, which is rich in lignocellulosic compounds, can be thermally converted into environmentally friendly bioherbicides. This study aimed to evaluate the potential of lignin-derived compounds from lignocellulosic waste as a bioherbicide and to identify an effective formulation for reducing the use of diuron-based herbicides in pineapple plantations. Pyrolysis was conducted at subcritical temperatures, and the resulting wood vinegar was analyzed by GC-MS to identify phenolic compounds. Pyrolysis at 250–300 °C produced phenolic compounds accounting for 40.20% of the chromatogram area, while pyrolysis at 400–450 °C produced 49.61%. The efficacy test included several treatments: pyrolysis products from 400–450 °C at 5% and 10%, pyrolysis products from 250–350 °C at 5% and 10%, diuron herbicide at 0.5 g/L, combinations of herbicide with two selected pyrolysis products, and a standard diuron application as the control. The results showed that wood vinegar produced at 400–450 °C and applied at 10% could suppress seed bank germination, with an effectiveness comparable to that of diuron at 0.5 g/L. This wood vinegar formulation was then used in a second efficacy test under pineapple cultivation conditions to assess whether diuron use could be reduced when combined with wood vinegar. The results indicated that combining 70% herbicide with 10% wood vinegar suppressed weed growth to a level similar to the 100% herbicide standard treatment. This study may serve as a reference for reducing the use of synthetic herbicides in pineapple cultivation.

Keywords: bioherbicide, hydrothermal carbonization, pre-emergence, pyrolysis carbonization, seed bank

Introduction

Indonesia's population continues to increase each year, although growth rates vary. According to the Statistics Indonesia (2022), Indonesia's population was projected to reach 275.77 million in 2022, an increase of 1.17% compared with 272.68 million in 2021. This population growth is accompanied by rising food consumption, particularly of vegetables and fruits. Survey results indicate that almost all Indonesians consume vegetables (94.8%), while 33.2% consume fruit. The average daily consumption is 70 g per person for vegetables and 38.8 g per person for fruit, totalling 108.8 g per person per day (Hermina & Prihatini, 2016).

Pineapple fruit is widely enjoyed because of its unique aroma, distinctive taste, and rich content of vitamins and minerals that are beneficial to health (Ali et al., 2020; Ismail et al., 2014). According to Statistics Indonesia (2021), pineapple production in Indonesia reached 2,886,417 tons in 2021, an increase of 439,174 tons compared with 2020. Pineapple is one of Indonesia's leading agricultural commodities and has a higher export volume than other major fruits such as bananas, oranges, durians, and mangoes.

Pineapple production generates waste amounting to up to 60% of the total fruit weight, consisting of peel (28.07%), core (8.81%), stem (2.25%), and crown (20.76%) (Singh et

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al., 2018). In recent years, numerous studies have investigated the use of pineapple waste to produce value-added products such as vinegar and wine, biofuel, biogas, organic acids, fiber, starch, and the enzyme bromelain (Sarangi et al., 2023). Increasing pineapple production to meet consumer demand requires attention to sustainability. Sustainability assessment encompasses three dimensions: social, economic, and environmental. Standards and indicators for sustainable agriculture are adjusted to the scale of agricultural practitioners, business size, and target market to create regulatory systems and certification schemes (Konefal et al., 2023). Applying sustainable agriculture with an eco-efficiency (EE) system can increase farmers' economic value and reduce the negative environmental impacts of cultivation by 4.7% (Phrommarat & Oonkase, 2021).

Pineapple biomass waste from the stem, peel, core, and crown has potential for utilization because it contains organic compounds such as cellulose, hemicellulose, and lignin (Asim et al., 2015). The pineapple biomass waste used in this experiment consisted of pineapple stems remaining after bromelain extraction. The weight of pineapple stems after peeling harvested plants can reach 0.6 kg per plant, and crude fiber, which represents potential waste, accounts for 56% of the total dry weight (w/w) (Nakthong et al., 2017). The lignin content in pineapple waste can be utilized to control plant pest organisms, including as a bioherbicide. This bioherbicide offers the potential to partially substitute the standard application of broad-spectrum synthetic herbicides, particularly diuron, which is commonly used in pineapple plantations.

Diuron is a systemic herbicide from the substituted urea class, widely used to control both perennial and annual broadleaf weeds and grasses in crops and non-crop areas. Its mode of action involves inhibiting photosynthesis by targeting Photosystem II (PS-II) in leaf chloroplasts. Specifically, diuron binds to the D1 protein within the PS-II complex, disrupting the electron transport chain that converts light energy into chemical energy. This blockage prevents the production of NADPH and ATP, which are essential for carbon fixation and nutrient synthesis, ultimately leading to chlorosis, oxidative damage, and plant death (Pest Control Products Board, 2022).

The continuous and increasing use of synthetic herbicides can be mitigated by blending them with bioherbicides, provided that the constituents exhibit neutral or, preferably, synergistic effects that better conserve the environment. This represents a fundamental stage to improve the quality of bioherbicides sourced from various biomass plants using different pyrolysis techniques, to gradually reduce the proportion of synthetic herbicide, and ultimately to achieve more environmentally friendly herbicide applications in pineapple plantations.

Lignin is one of the most abundant biopolymers and provides structural integrity and mechanical resistance to plant tissues (Amores-Monge et al., 2022; Tobimatsu & Schuetz, 2019). The composition of cellulose, hemicellulose, and lignin varies among different pineapple plant organs and among different pineapple waste biomass conditions (Ankona et al., 2023; Mansor et al., 2019; Pereira et al., 2022). Lignin can be pyrolyzed to obtain phenolic compounds. Phenolic compounds from several plants have been identified as bioherbicides with different components and concentrations (Tigre et al., 2015; Tong et al., 2021). Bioherbicides offer advantages such as degradability, low residue, and minimal environmental contamination (Campos et al., 2019; Soto-Maldonado et al., 2022). The pyrolysis of pineapple waste biomass must be conducted carefully to obtain an optimal amount of phenolic compounds. According to Agusta et al. (2022), Hung et al. (2022), and Hung et al. (2023), the pyrolysis process to produce phenolic compounds can be carried out at a heating temperature of at least 300 °C under low-oxygen conditions.

The results of this study are expected to determine the response of lignin-derived compounds with potential as bioherbicides from pineapple waste and to identify mixed formulations between synthetic herbicides and bioherbicides derived from pineapple waste

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that can reduce synthetic herbicide use while maintaining weed control efficacy.

Material and Methods

Preparation of Wood Vinegar Solution by Pyrolysis Process

Wood vinegar was produced from pineapple stem waste remaining after bromelain extraction using a pyrolysis machine. The raw material was first dried in an oven at 100 °C until the moisture content reached approximately 20%. For pyrolysis at 200–250 °C and 250–300 °C, the dried material was ground to approximately 30 mesh. For each pyrolysis run, 2 tons of biomass were required to produce 20 L of wood vinegar (a 1:100 yield ratio). The fine biomass was mixed with water at a 1:10 ratio (biomass:water). Nitrogen gas (N_2) was then flushed into the closed reactor and flushed out to remove oxygen (O_2), ensuring that the remaining 45 L of reactor capacity contained only inert N_2 gas. The reactor was subsequently evacuated using a vacuum pump. Heating was maintained at the target temperature for 60 min, and condensation occurred in a closed system to collect wood vinegar.

For pyrolysis at 400–450 °C, 2 tons of material were also required to yield 20 L of wood vinegar (1:100 ratio). Water was gradually added throughout the pyrolysis process, and the condensate was collected once the temperature reached 100 °C. The wood vinegar products were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS). In the first trial, the wood vinegar product with the highest phenolic content was selected for efficacy testing.

Wood Vinegar Efficacy Test as Bioherbicide on Seed Bank with Bio-Assay Test

The efficacy experiment of wood vinegar used a completely randomized design. Wood vinegar was applied to a weed seed bank obtained from the Cikabayan experimental station area of IPB University in Bogor. The soil containing the weed seed bank was collected from the upper 0–20 cm layer under a multiyear annual crop rotation system. This location had previously been used for various annual field crops but was not under monoculture at the time of sampling. The application to the weed seed bank aimed to assess the effectiveness of weed control at the pre-emergence growth stage.

In the early-stage efficacy experiment, there were 7 treatments with 3 replications:

1. Wood vinegar (250–300 °C) at 1 ml/L (10% v/v)
2. Wood vinegar (250–300 °C) at 0.5 ml/L (5% v/v)
3. Wood vinegar (400–450 °C) at 1 ml/L (10% v/v)
4. Wood vinegar (400–450 °C) at 0.5 ml/L (5% v/v)
5. Diuron herbicide at 0.5 g/L (0.5% w/v)
6. Combination of wood vinegar (400–450 °C) 10%, wood vinegar (250–300 °C) 10%, and diuron herbicide 0.5 g/L
7. Diuron herbicide 0.5 g/L alone (standard control)
8. Control with no application

The weed seed bank was placed in trays measuring 328 mm × 284 mm × 50 mm. The soil used as the source of the weed seed bank was collected from the 0–10 cm layer, homogenized, and distributed equally among experimental units at 1.8 kg per unit. The weed species present at the Cikabayan experimental station included *Borreria alata, Rubia redsaltata, Asystasia gangetica, Eleusine indica, Ageratum conyzoides, Axonopus compressus, Cyperus* sp.*, Ottochloa nodosa, Cleome rutidosperma, Phyllanthus niruri, Synedrella* sp.*,* and *Amaranthus spinosus*. The application spray volume corresponded to 3000 L/ha. A small 1-liter handsprayer was used to spray, with a spray volume of 0.32 L per seed bank bed. Weed dominance was analyzed based on the number of weeds, weed species, and weed dry weight. The number of weeds was observed at 3–6 weeks after treatment.

Data were statistically analyzed using the *F* test (ANOVA) at the 5% significance level,

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using Microsoft Excel and R-Studio software. Treatments that showed a significant effect at the 5% level were further compared using Duncan's multiple range test (DMRT) to determine differences between treatments.

Efficacy Test of Wood Vinegar as Bioherbicide at Post-Planting Stage in Pineapple Plantation

The efficacy experiment in pineapple plantations used a randomized complete block design. The wood vinegar product selected for this field test was based on the results of the previous bio-assay experiment. The field experiment was conducted in a pineapple (Ananas comosus) plantation in Lampung, Indonesia. Treatment application was conducted 1 week after planting.

The experimental unit plot measured 7 m × 5 m, with pineapple planting material consisting of healthy crowns or slips spaced at 55 cm × 25 cm. In this efficacy test, there were 6 treatment levels with 4 replications:

1. Diuron herbicide at 0.5 g/L (0.5% w/v)
2. Combination of diuron herbicide at 0.45 g/L and wood vinegar bioherbicide (400–450 °C) 10% (v/v)
3. Combination of diuron herbicide at 0.35 g/L and wood vinegar bioherbicide (400–450 °C) 10% (v/v)
4. Combination of diuron herbicide at 0.25 g/L and wood vinegar bioherbicide (400–450 °C) 10% (v/v)
5. Wood vinegar bioherbicide (400–450 °C) 10% (v/v) alone
6. Control with no application

Weed control was applied 1 week after planting at a spray volume of 3000 L/ha.

Toxicity effects on pineapple plants were observed through stem diameter, leaf length, leaf width, plant height, plant wet weight, leaf greenness, and leaf number. Stem diameter and plant wet weight were observed at the beginning and end of the experiment. Leaf length, leaf width, and plant height were observed monthly from 1 month after treatment (MAT) until 4 MAT. The number of leaves was observed at 4 MAT. Leaf greenness was measured using the Soil–Plant Analysis Development (SPAD) meter at 1 day after treatment (DAT), 3 DAT, 1 week after treatment (WAT), 2 WAT, and 1 MAT.

The effect of treatments on weed growth was observed monthly from 1–4 MAT using weed density (weed crown area) as the parameter. Dominance level was assessed at 4 MAT using weed number, weed dry weight, and weed frequency. Weed sampling for vegetation analysis to determine weed dominance (SDR) was conducted using purposive sampling and a 1 m × 1 m quadrat method, with 2 quadrats per experimental unit.

Results and Discussion

Seedbank Bioassay Test

Wood vinegar Compound Components

Increasing the pyrolysis temperature affects the chemical composition of wood vinegar. At pyrolysis temperatures of 250-300 °*C* tends to produce a variety of complex organic compounds. There are two dominant groups of compounds produced at each temperature treatment: aldehyde and ketone groups, and phenol and phenolic ether groups (Table 1). Groups of phenolic compounds and phenolic ethers tend to be produced as the temperature increases. The process of structural changes that occur to increase phenol production consists of: 1) a decrease in aliphatic carbon structure, 2) a decrease in methoxy carbon structure due to decomposition of ether bonds such as α-O-4 and β-O-4, 3) an increase in aromatic carbon structure, 4) a decrease in carbonyl carbon structure, such as CO_2 (Lu & Gu, 2022).

Bioassay of wood vinegar as a bioherbicide against the seed bank was conducted using wood vinegar products produced at 400-450 °C and 250-300 °C. The test was conducted with consideration of the phenol content produced. Bioherbicides containing phenolic compounds have the potential to suppress germination of broadleaf weed seeds, including *Echium*

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vulgare, *Rumex acetosa*, *Scabiosa triandra*, *Sinapis arvensis*, *Sonchus asper*, *Centaurea melitensis,* and *Conyza canadensis* (Saludes-Zanfaño et al., 2022).

Efficacy of Wood Vinegar as Bioherbicide on Seed Bank

Wood vinegar efficacy testing was carried out on pyrolysis products produced at 400–450 °C and 250–300 °C. The application of wood vinegar, herbicide, or a combination of both significantly controlled weed growth (Table 2). There was a difference in results between combined treatments and non-combined treatments. However, there was no significant difference between single applications of the herbicide (diuron) and the bioherbicide (wood vinegar), indicating the potential of wood vinegar as a bioherbicide to replace synthetic herbicides.

The comparison between wood vinegar products produced at 400–450 °C and those produced at 250–300 °C showed a significant difference. For wood vinegar produced at 250–300 °C, the comparison between 5% and 10% concentrations showed a significant difference at both the beginning and end of the observation period. In contrast, wood vinegar produced at 400–450 °C showed no significant difference between 5% and 10% concentrations.

The wood vinegar product and application concentration for further efficacy testing in pineapple cultivation were selected based on formulations that provided the same weed-control effect as conventional herbicide products. Wood vinegar produced by pyrolysis at 400–450 °C exhibited the same suppressive effect as herbicide products and their combinations on weed seed germination. The efficacy of wood vinegar as a bioherbicide can suppress weed growth by more than 50% at application concentrations (v/v) above 5% (Hagner et al., 2020). In contrast, application concentrations below 5% can stimulate seed germination. For example, application of wood vinegar at a concentration of 1% has been shown to stimulate the growth of bok choy seeds (Ju et al., 2021).

Efficacy Test on Pineapple Plantation

Analysis of Herbicide Toxicity on Pineapple

The application of herbicides and wood vinegar for weed control in pineapple environments did not have a toxic effect on pineapple growth and development based on the parameters of leaf width, leaf length, leaf chlorophyll level, stem diameter, plant wet weight, and number of leaves (Figures 1, 2, and Table 3).

Table 1

Chemical Characterization of Wood Vinegar Derived from Pineapple Stem Waste Using GC-MS

Organic Compounds	% Area		
	200-250 °C	250-300 °C	400-450 °C
Aldehydes and ketones	9.52	21.02	20.40
Phenols and phenolic ethers	18.48	40.20	49.61
Aniline	2.66	-	-
Heterocyclic	-	9.87	7.65
Hydrocarbons	-	6.13	-
Alkaloids	-	5.24	-
Amines	-	1.27	-
Terpenoids	-	1.45	-
Alcohols	-	3.00	-
Esters	-	7.80	1.47
Amino acids	-	-	4.45

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Table 2

Efficacy of Wood Vinegar Products Against the Seed Bank

Treatments	Weed seedling number/tray	
	3 WAT	6 WAT
Wood Vinegar 250-300 °C; 0.5 ml/L	6.67[bc]	10.00[b]
Wood Vinegar 250-300 °C; 1 ml/L	2.00[bc]	9.33[b]
Wood Vinegar 400-450 °C; 0.5 ml/L	8.33[bc]	3.67[bc]
Wood Vinegar 400-450 °C; 1 ml/L	11.33[b]	1.00[c]
Diuron Herbicide 0.5 g/L	1.00[c]	1.00[c]
Herbicide + Wood vinegar 400-450 °C;1 ml/L + Wood vinegar 250-300 °C;1 ml/L	5.00[bc]	1.00[c]
Diuron standard application (control)	12.33[a]	1.02[a]
Significance	**	***

Notes. WAT= Week after treatment. Values followed by the same letter in the same column are not significantly different according to the 5% DMRT.

Figure 1

The Effects of the Synthetic Herbicide and the Bioherbicide Ratio on Pineapple Leaf Size

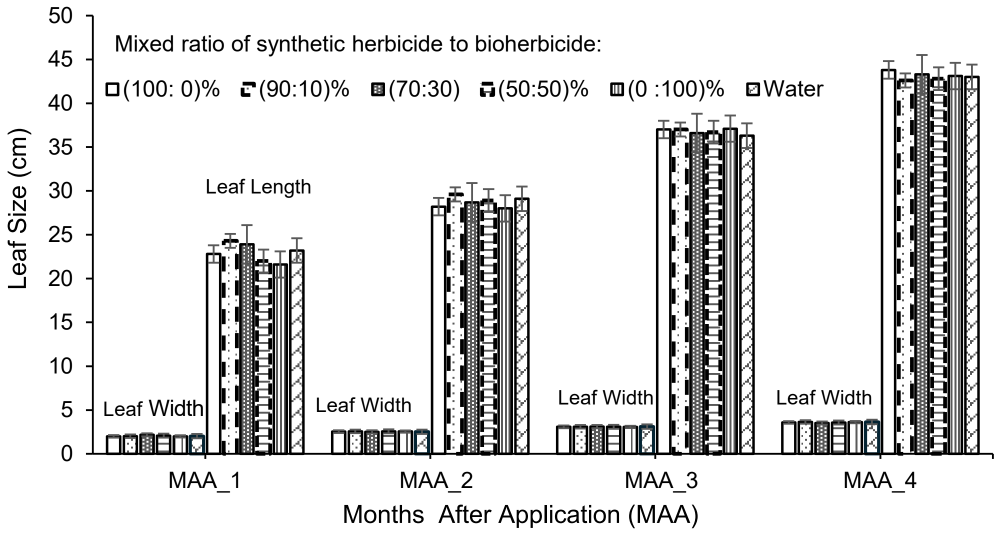

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Figure 2

The Effects of the Synthetic Herbicide and the Bioherbicide Ratio on the Pineapple Leaf Content from 1 to 30 Days After Application

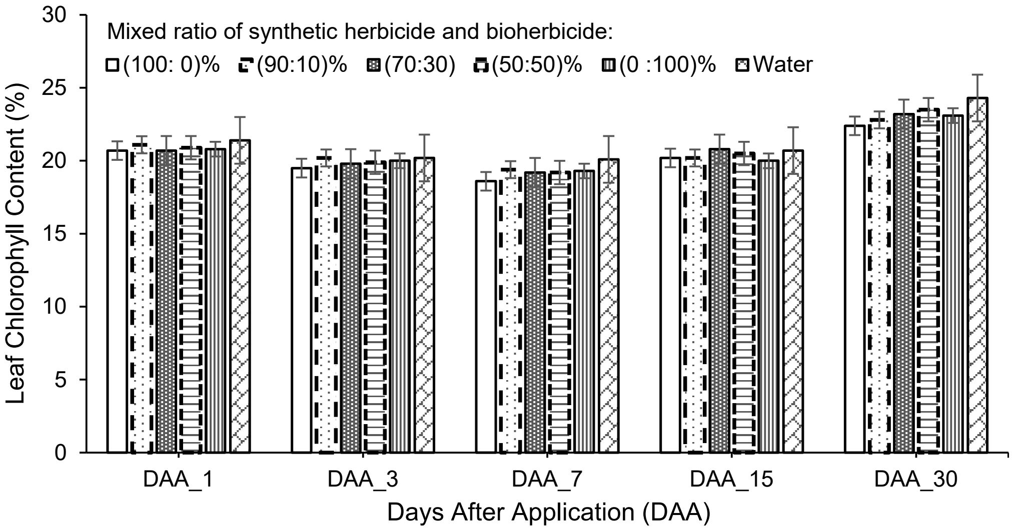

Table 3

Pineapple Plant Growth and Development 4 Weeks After Planting

Treatments	Stem diameter	Plant fresh weight (g)	Number of leaves
Herbi[1] 100%	2.72 ± 2.01	220.11 ± 99.90	25.23 ± 3.83
Herbi 90%+10% Bio(400-450) °C	2.17 ± 1.48	252.69 ± 111.31	25.85 ± 5.04
Herbi 70%+30% Bio(400-450) °C	3.04 ± 2.44	246.37 ± 133.85	25.83 ± 5.70
Herbi 50%+50% Bio(400-450) °C	2.33 ± 2.03	283.11 ± 128.38	26.78 ± 5.88
Bioherbicide 100% (400-450) °C	2.85 ± 2.27	255.38 ± 123.05	25.35 ± 4.82
Water treatment as control	2.35 ± 1.76	235.42 ± 103.93	25.68 ± 4.29

Notes. [1]Herbi: the standard diuron herbicide, Bio: wood vinegar solution based, Control: no application of Herbi nor Bio, the blending composition was based on a spraying volume of 3000 L/ha.

Pineapple plant growth and development were similar across all treatments. Previous research on weed control in pineapple also reported a similar response, indicating that weed control had no significant effect on pineapple growth and development up to 6 weeks after treatment (WAT) (Tajudeen et al., 2020). According to Pegoraro et al. (2014), vegetative biomass production in pineapple plants up to 463 days after planting (DAT) ranged from 21% to 40%, with an average leaf length growth rate of 2.40 mm per day (0.16 cm per day) and an average leaf width growth rate of 0.21 mm per day; these findings suggest that weed control had minimal effect on pineapple growth and development under the conditions studied.

Toxic effects in pineapple plants were observed at a diuron application rate of 3 kg/ha, with fluorescence ratio (Fv/Fm) values of 0.765 at 7 days after application (DAP) and 0.690 at 21 DAP (Reis et al., 2024). An Fv/Fm value below the optimal range (Fv/Fm of 0.800 ± 0.05) indicates that the photochemical system in pineapple plants has been disrupted (Bolhar-Nordenkampf et al., 1989).

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Effectiveness of Control Against Weed Growth

The summed dominance ratio (SDR) is a parameter used to measure the relative dominance of a weed species in a plant community. *Ottochloa nodosa* weeds (44.5%) were the dominant weeds in the pineapple cultivation environment at 4 months of plant age (Table 4). Grass weeds (*Ottochloa nodosa*, *Digitaria ciliaris*, *Paspalum dilatatum,* and *Borreria alata)* with a total SDR of 80.43% were the most dominant weed group when compared to broadleaf and grass groups based on the classification of weed leaf morphology. Weed dynamics (species diversity and community uniformity) at the same location were influenced by control techniques and materials used (chemical control). Manual weed control had minimal impact on the dominant weed community. but significantly affected the weed composition. Long-term use of pre-planting herbicides and manual weeding maintained the weed community in a stable structure with lower losses (Gao et al., 2020).

Treatment with herbicides, and a combination of herbicides and wood vinegar, can reduce weed dominance compared to wood vinegar application or no control. The combination of 70% herbicide with wood vinegar bioherbicide showed the same response as a

Table 4

Weed Dominance in the Pineapple Cultivation Environment 4 Weeks After Treatment

Species	Summed dominance ratio (%)
Digitaria ciliaris (Retz.) Koeler	32.38%
Ottochloa nodosa (Kunth) Dandy	44.50%
Fimbristylis miliacea (L.) Vahl	5.90%
Cyperus esculentus L.	7.50%
Cyperus rotundus L.	3.81%
Paspalum dilatatum Poir.	2.68%
*Ageratum conyzoide*s L	1.48%
Borreria alata (Aubl.) DC.	0.87%
Kyllinga nemoralis (J.R.Forst. & G.Forst.) Dandy ex Hutchinson & Dalziel)	0.88%

Note. Values followed by the same letter in the column are not significantly different according to the 5% DMRT.

Table 5

Effect of Weed Control Treatments on Weed Growth 4 Weeks After Treatment

Treatments	Weight (g)	Frequency	Weed density (%)	Summed dominance ratio (%)
Herbi[1] 100%	6.75^{c}	1.0^{bc}	0.65^{c}	6.68^{b}
Herbi 90% + Bio	2.75^{c}	0.5^{c}	0.23^{c}	2.67^{b}
Herbi 70% + Bio	2.25^{c}	0.5^{c}	0.48^{c}	2.13^{b}
Herbi 50% + Bio	23.25^{bc}	0.5^{c}	1.63^{bc}	7.58^{b}
Biohercide	58.25^{ab}	2.3^{ab}	3.96^{ab}	29.05^{a}
Control (water)	94.75^{a}	3.0^{a}	8.93^{a}	35.56^{a}
Significance	**	**	**	***

Notes. [1]Herbi indicates the application of diuron, a standard synthetic herbicide. Values followed by the same indexed letter in the same column are not significantly different according to the 5% DMRT.

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100% herbicide application, as indicated by SDR parameters and weed density. This indicates that wood vinegar can substitute for herbicides up to 30% with the combination (Table 5).

Effective weed control management needs to consider the dominant weed species and the critical period of weed interference. The critical period represents the time when negative interactions between weeds and crops are most pronounced and indicates the optimal window for weed control (Marques et al., 2017). Weeds that grow before or after this critical period do not significantly affect crop yields (Tiririca, 2000). Therefore, determining the start and end of the critical period depends on the level of acceptable crop loss (Knezevic & Datta, 2015). In pineapple, the critical period of weed presence that reduces productivity by 5% occurs at 14–259 days after stem planting (DAST), while the critical period that reduces productivity by 10% occurs at 51–204 days after transplanting (DAT). Weed competition with pineapple plants during the production cycle can reduce yields by 69.50% compared to plots with weed control during the same period (de Oliveira et al., 2021).

Conclusions

Degradation of lignocellulosic waste by pyrolysis at 400–450 °C produced the highest phenolic compound content (49.61%) and provided the same suppressive effect on weed seed germination (seed bank) as diuron herbicide at 0.5 g/L when applied at a concentration of 1 ml/L (10% v/v). The combination of wood vinegar produced at 400–450 °C at 10% concentration with the synthetic herbicide diuron effectively controlled weed growth, reducing diuron use by up to 30% without compromising weed control.

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