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EFFECT OF LIQUID SMOKE FROM LIGNOCELLULOSE WASTE ON THE GROWTH OF *Orthosiphon aristatus* (Blume) Miq. UNDER A HYDROPONIC WICK SYSTEM

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ABSTRACT

Liquid smoke is a by-product of the lignocellulosic pyrolysis process and has long been used for various purposes, including as ~~a~~ plant growth stimulant. The use of liquid smoke for the growth of medicinal plants, especially *Orthosiphon aristatus* (Blume) Miq., is yet to be widely carried out. This study aimed to determine the effect of liquid smoke concentration on the phytotoxicity of cat whiskers, the growth response of cat whiskers in a wick hydroponic system for sustainable production practices, and the phenol and acetic acid contents in the liquid smoke solution. The measured growth response parameters included leaf, stem, root, total biomass, plant height, root length, stem diameter, and sinensetin levels. This study used liquid smoke collected using the stratification technique at 200 ^{0}C and 400 ^{0}C from three raw waste materials: pine wood, teak wood, and bamboo. The concentrations of liquid smoke in the hydroponic solution media were 0.25%, 0.5%, 1%, and 2%. The results showed that liquid smoke at 400 ℃ and concentrations of ≥3% caused significant phytotoxicity, including leaf wilting, necrosis, chlorosis, and plant death, owing high phenol and acetic acid levels. Furthermore, Andong bamboo liquid smoke (ABLS) at a concentration of 0.25% collected at 200 ^{0}C generally gave better results for the growth of the cat whiskers plant on hydroponics. It increased sinensetin content compared to the control and other treatments. These findings suggest that stratifying pyrolysis temperatures and properly diluting liquid smoke can be used as growth stimulants while minimizing phytotoxicity.

Keywords: cat whiskers, hydroponic, liquid smoke, plant growth, pyrolysis

INTRODUCTION

Industrialization in various sectors of life has increased the chemical load of natural ecosystems, such as pesticides, fertilizers, and chemical growth regulators, which are used extensively. However, despite their advantages, these synthetic chemical materials have many adverse effects on human health and the environment (Kalloo *et al*., 2018; Kumar *et al*., 2020; Buckley *et al*., 2021).

This prompted an international agreement to restrict the use of chemicals in production, especially synthetic chemical pesticides, for pest and disease control in agriculture, forestry, and plantations. The European Union has made a political decision to raise environmental awareness through a directive with the code 2009/128/EC, which encourages the development of crop protection and integrated pest management (IPM) in the EU. One is a biological control technology that minimizes environmental and human health risks, such as natural ingredients or botanical products (Helepciuc and Todor, 2021).

Natural ingredients have been extensively researched and have provided good results. Some plants, such as onions and bamboo shoots, are effective growth boosters, including auxins and gibberellins (Hasibuan *et al*., 2020; Lukman *et al*., 2022). Liquid smoke from the carbonization of lignocellulose is another natural product of renewable materials. Liquid smoke is a by-product of lignocellulose carbonization/pyrolysis. Previous studies have reported that liquid smoke can be used as a plant growth stimulant for paddy, horticulture, and forestry plant seeds (Chen *et al*., 2016; Wang *et al*., 2019) and as a food preservative (Faisal and Gani, 2018; Desvita and Faisal, 2020), deodorizing or coagulant agents in rubber processing, biopesticides, antibacterial, and antifungal agents (Li *et al*., 2018; Chukeatirote and Jenjai, 2018; Suresh *et al*., 2019).

Several researchers have studied the effect of liquid smoke on plant growth and seed germination and reported varying results according to liquid smoke concentration, type of seed, and type of plant (Wang *et al.*, 2019; Lu *et al.*, 2019). Liquid smoke, as a plant growth stimulant, significantly increases the weight of fresh cayenne fruits (Yuniwati and Lestari, 2020). It also increases seed germination, seedling growth, root volume, and total root length in tomato plants (Ofoe *et al.*, 2022). In rapeseed plants, liquid smoke encourages or stimulates growth by increasing the number of pods and leaf area index (Zhu *et al.,* 2021). Meanwhile, in onions (*Allium cepa* L.), liquid smoke promotes tissue-specific effects on onion metabolism, involving primary and secondary metabolites, thus significantly increasing the enzyme-inhibiting and antioxidant properties of onions (Zhang *et al*., 2023). These results suggest the potential for increased production of secondary metabolites or active substances in medicinal plants treated with liquid smoke. However, according to literature searches, research on medicinal plants, particularly cat whiskers treated with liquid smoke remains limited.

Cat whiskers (*Orthosiphon aristatus*) are well-known as a traditional medicine already used in several countries, such as Indonesia, Malaysia, Myanmar, Thailand, Vietnam, South Korea, and Japan. Some health benefits of plant leaves have been reported, such as antidiabetic, hypertension, diuretic, hepatitis, antipyretic, gout arthritis, kidney tonic, and detoxification effect. The active substance in the cat whiskers plant is sinensetin, a polymethoxylated flavonoid (Abdullah *et al*., 2020).

The cultivation of cat whiskers is generally carried out using soil in gardens or farmland for large production capacities. People with limited land or space can engage in cultivation through hydroponics. The hydroponics of cat whiskers using liquid fertilizer media mixed with liquid smoke are yet to be studied. Therefore, this study aimed to determine the effects of liquid smoke from teak wood, pine wood, and bamboo waste (produced at 200 ^{0}C and 400 ^{0}C) on the phytotoxicity of cat whiskers plants, levels of acetic acid and phenol, and the effect of adding liquid smoke to the wick hydroponic system media on the growth of cat whiskers.

MATERIALS AND METHODS

The study was conducted in a greenhouse at The Ministry of Environment and Forestry, Bogor, West Java, Indonesia. The average temperature, light intensity, and air humidity were 32.2 ^{0}C, 33.124 Lux, and 69.2 % RH, respectively.

Liquid smoke preparation: Teak wood liquid smoke (TWLS), pine wood liquid smoke (PWLS), and Andong bamboo liquid smoke (ABLS) are produced using the pyrolysis temperature stratification method. This study used liquid smoke produced at 200 °C and 400 °C, based on low and high values of phenol and acid (Wibowo *et al*., 2023). AAS analysis was used to determine the nutrient content of cat whiskers leaves. HPLC analysis of sinensetin content in cat whiskers leaves and Py-GCMS analysis of chemical compounds. Crude liquid smoke was stored for one month, and the tar was separated. It was then diluted by adding distilled water to concentration of 0.25, 0.5, 1, 2, 3, 5, and 7%. Subsequently, the acetic acid and phenol concentrations were analyzed according to the method described by Wibowo *et al*. (2023).

Plant preparation: The cat whiskers (*Orthosiphon aristatus* (Blume) Miq. White varieties) stem cuttings were obtained from farming in Wates Jaya Village, Cigombong District, Bogor Regency, West Java, Indonesia, sourced from 2-year-old plants. The length of the stem cuttings was uniform, at 20 cm. The stem cuttings were planted in polybags (ratio of soil: manure: husk 1:1:1). There were two groups of planting media: soil and hydroponic. Soil media are used for phytotoxicity treatment, and nutrient solution media were used for hydropic treatment. For phytotoxicity analysis, plants were maintained until they were two months old. Meanwhile, the same source of stem cuttings was used for hydroponic treatments, which were planted in polybags until the roots and initial leaves emerged (10-15 days) and then transferred to hydroponic media.

Treatment: The phytotoxicity treatments used TWLS, PWLS, and ABLS at concentrations of 0.25, 0.5, 1, 2, 3, 5, and 7%, respectively. A liquid smoke solution (100 mL) was used for plants according to the concentration of the liquid smoke treatment, where 50 mL was poured onto the media in the polybag, and 50 mL was sprayed onto the leaves and stems. Phytotoxic symptoms were observed daily for up to 15th day after treatment. Leaves that cause phytotoxic symptoms (wilting/chlorosis) were divided into five scores: 0 (no symptoms), 1 (1-3 leaves), 2 (4-6 leaves), 3 (7-9 leaves), 4 (more than 9 leaves), and 5 (plant dead). The severity of phytotoxicity symptoms was ranked after application with a score of 0-1 (no symptoms), low (1.1-2.5), medium (2.6-4.0), and high (4.1-5.0) (Aisyah *et al*., 2018).

The hydroponic fertilizer (AB Mix) is a fertilizer starter solution consisting of formulas A and B. Formula A contains potassium nitrate, calcium ammonium nitrate, and Fe-chelate. Formula B contains ammonium sulfate, ammonium heptamolybdate, potassium dihydrophosphate, potassium sulfate, magnesium sulfate, manganese(II) sulfate, copper, and zinc sulfate. The initial AB solution was 1200 ppm (TDS meter measurement). Hydroponic media nutrient solutions were prepared by mixing water, hydroponic fertilizer, and liquid smoke to obtain hydroponic media solutions with liquid smoke concentrations of 0.25, 0.5, 1, and 2%. The choice of concentration was based on the results of phytotoxicity analysis, where concentrations ≤2% did not cause plant phytotoxicity. A wick hydroponic system is used in this study. The wick system delivers water and nutrients from the container to the plant through wick strips of cloth to absorb the nutrients to the plant roots. During the three-month plant maintenance period, we provided media solutions with liquid smoke three times: at the start of the treatment, one month later, and two months later. Plants were harvested at three months of age and analyzed.

The parameters analyzed were plant height, fresh weight of leaves, stems, and roots, increase in total biomass weight (leaves, stems, and roots), root length, plant nutrient testing, and sinensetin content (Hossain and Ismail, 2016). Plant height and root length were measured using tape, the weight of plant parts was measured using an Ohaus digital scale, and the stem diameter was measured using a digital caliper. The total biomass was the sum of the weights of the leaves, stems, and roots. The total nitrogen content in cat whiskers leaves was measured using the Kjeldahl method (Servin *et al*., 2021). Phenol measurements were based on the SNI 01-3711-1995 method, and acetic acid was measured using the Folin-Ciocalteu method (Wibowo *et al*., 2023).

Plant nutrient analysis: For nutrient analysis, 0.2 g of dried fine powder samples were put into the destruction tube, and 2 mL of acidic solution (a mixture of nitric acid, perchloric acid, and sulfuric acid) was added. After 24 h, the destruction tube was heated to 200 °C. The solution was then diluted to a volume of 10 mL and homogenized. Total N was analyzed using the micro-Kjeldahl method. Total P was analyzed using a spectrophotometer at a wavelength of 693 nm. K, Mg, and Ca were analyzed using AAS.

Measurement of sinecetin leaf content was carried out three months after hydroponic planting based on the best growth results at 200 °C and 400 °C (0.25%) liquid smoke concentrations. Sinensetin content was analyzed by high-performance liquid chromatography (HPLC). A powdered sample (2 g) was added to 100 mL of methanol and shaken for 4 h. The extract was then inserted into a evaporator flask and applied to the remaining 5 mL. The extracts were dissolved in 10 mL of a solvent (MeOH:water = 6:4). After filtration, the sample extract was injected into an HPLC system (Febjislami *et al*., 2019).

Statistical analysis: Experimental data were analyzed by two-way analysis of variance (ANOVA). Means of treatment were compared by the Duncan test at 5% (p <0,05), assisted by the SPSS 27 statistical software.

RESULTS

Phytotoxicity of liquid smoke: Phytotoxicity analysis on cat whiskers using teak wood liquid smoke (TWLS) 200 ^{0}C, at concentration up to 5% showed no phytotoxic symptoms (score 0.0) on the 15th day. However, at a concentration of 7%, phytotoxicity symptoms occurred with a score of 3.8 (medium). Pine wood liquid smoke (PWLS) 200 ^{0}C at concentrations of 0.25, 0.5, 1, and 2% did not cause phytotoxic symptoms (score 0.0). However, phytotoxic symptoms occurred at concentrations of 3, 5, and 7%, with scores of 2.6, 4 and 4.8 (medium and high symptoms) (Table 1). Andong bamboo liquid smoke (ABLS) 200 ^{0}C, at concentrations of 0.5, 1, and 2% did not cause phytotoxic symptoms (score 0.0). At concentration of 3% and 5%, phytotoxic occurred, with score of 0.2 and 0.8, respectively. These results show that PWLS at 200°C is safe to use up to a concentration of 2%, whereas liquid smoke from teak and bamboo is safe to use up to 5%.

The application of liquid smoke at 400 ^{o}C (TWLS, PWLS, and ABLS) at concentrations of 0.25, 0.5, and 1% did not show any leaf phytotoxic symptoms. In contrast, the 2% liquid smoke concentration produced scores of 0.6, 0.8 and 0.6 (Table 1). This values is still considered safe and is categorized as asymptomatic because it is in the range of 0-1 (Aisyah *et al*., 2018). However, at a concentration of 3%, phytotoxic symptoms were observed in the low and medium category but did not result in plant death. Concentrations of 5% and 7% were in the high category and caused severe phytotoxicity, namely, the leaves became necrotic (burn), dried out, fell of, the stem dried out, and died on the 15th day after application of liquid smoke.

Table 1. Evaluation of liquid smoke for its phytotoxicity effect on cat whiskers plant.

Sample	Temp. (℃)	Dosage						
		0.25%	0.5%	1%	2%	3%	5%	7%
TWLS	200	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	3.8±0.8 ^{e}
	400	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0.6±0.6 ^{ab}	1.6±0.9 ^{c}	3±1.0 ^{d}	5±0.0 ^{f}
PWLS	200	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0.8±0.5 ^{b}	4±0.0 ^{e}	4.2±0.5 ^{e}
	400	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0.8±0.5 ^{b}	3±1.0 ^{d}	4.8±0.5 ^{f}	5±0.0 ^{f}
ABLS	200	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	1±0.0 ^{b}	3.2±0.8 ^{d}
	400	0±0.0 ^{a}	0±0.0 ^{a}	0±0.0 ^{a}	0.6±0.6 ^{ab}	1.8±0.5 ^{c}	4.2±0.5 ^{e}	4.8±0.5 ^{f}

Remark: Values represent the mean of five replicates ± standard deviation (SD). Numbers followed by the same letter indicate insignificant difference based on the 5% DMRT test. TWLS: Teak Wood Liquid Smoke, PWLS: Pine Wood Liquid Smoke, ABLS: Andong Bamboo Liquid Smoke

The growth response of Cat whiskers plants: The results of hydroponic research on cat whiskers plants show that TWLS, PWLS, and ABLS liquid smoke treatment at a concentration of 0.25% significantly stimulated the growth, increasing plant height, root length, wet weight of biomass compared to the control. However, at a concentration of 1%, plant growths were stunted, and at 2% ABLS and PWLS both at 200 ^{0}C and 400 ^{0}C caused plant death (except for TWLS treatment at 200 ^{0}C).

The highest leaf weight was obtained in the ABLS treatment at 200 ^{0}C with a concentration of 0.25% (17.71±6.29 g). This was not significantly different from the leaf weight of 14.43±11.63 g from TWLS at 200 ^{0}C with a 0.25% concentration. However, both were significantly higher than the controls (7.17±3.49 g). The lowest leaf weight produced by ABLS at 400 ^{0}C with a 1% concentration of 0.78±0.17 g. The highest stem weight was detected in TWLS treatments at 200 ^{0}C with a 0.25% concentration of 10.56±8.88 g. This result was not significantly different from the stem weight of 8.40±2.74 g from the ABLS treatment at 200 °C with a 0.25% concentration. However, it was significantly higher than the control, which had a stem weight of 4.68±1.66 g. The lowest stem weight was produced by the ABLS treatment at 400 °C with a 1% concentration of 0.78±0.42 g. Meanwhile, the highest root weight was obtained from ABLS treatment at 200 ^{0}C at 0.25% (6.97±2.20 g) and the lowest was observed from ABLS treatment at 400 ^{0}C, a concentration of 1% (0.45±0.26 g) (Table 2).

Figure 1. Symptoms of phytotoxicity in cat whiskers plants. a. Wilted leaves, b. Chlorosis leaves, c. Plant death.

Total biomass weight was calculated as the weight of the leaves, stems, and roots after harvest minus the initial weight of the plant. Table 2 shows that the highest increase in biomass weight was obtained with ABLS treatment at 200 ^{0}C with a concentration of 0.25%, i.e., 32.16 g, and the lowest was produced from ABLS at 400 ^{0}C with a concentration of 1%, i.e., 1.27 g. The results of the variance analysis indicated that liquid smoke treatment had a significant impact on biomass weight. However, Duncan's follow-up test revealed that several treatments fell into the same group, indicating that they were not significantly different from each other.

ABLS treatments at 200 ^{0}C in a concentration of 0.25% increased plant height by 42.14 cm, followed by TWLS by 32.8 cm and PWLS by 23.66 cm. These results significantly differed from those of the control treatments, with an increase of only 23.3 cm. The lowest increase in plant height was observed in ABLS at 400 ^{0}C, in concentration 1%, i.e., 7.0 cm, lower than that of the control treatment. Meanwhile, at a concentration of 2% (except for teak liquid smoke at 200 ^{0}C), liquid smoke causes plant death. The symptoms of dead plants included dark brown leaves (most have fallen off), black and hardened stems, and black and rotting roots. The highest increase in plant root length was calculated detected in the TWLS treatments at 200 ^{0}C and a concentration of 0.25%, i.e., 36.26 cm, which was higher than that of the control (24.9 cm). The lowest increase in root length (1 cm) resulted from the PWLS treatment at 400 ^{0}C. Stem diameter measurements showed that TWLS treatment at 200 ^{0}C (0.25%) significantly increased the stem diameter, i.e. by 2.1 mm. These values were higher than those of the control treatment, which had an additional diameter of 1.18 mm. The lowest increase in diameter was found in the 400 ^{0}C pine liquid smoke treatment at a concentration of 1% (0.72 mm). Treatments with a concentration of 2% (except TWLS, 200 ^{0}C) caused plants death.

Sinensetin content was analysed in harvested leaves treated with liquid smoke at 200 ^{0}C and 400 ^{0}C with a concentration of 0.25%. The highest sinensetin content was found in leaf samples treated with ABLS at

200 ^{0}C of 0.293 mg/g, which was higher than that of the control of 0.172 mg/g, while the lowest sinensetin content was found in the PWLS treatment at 400 ^{0}C (Table 2).

Phenol, acetic acid, and nutrient: Liquid smoke phenol analysis showed a decrease in phenol and acetic acid levels in liquid smoke dilution compared with crude liquid smoke. The lowest phenol content was found in TWLS 200 ℃ at a concentration of 0.25% (0.00024%), and the highest content was found in PWLS 400 ℃ at a concentration of 7% (0.1%) (Figure 2a). Meanwhile, the lowest acetic acid content was also found in TWLS 200 ℃ at a concentration of 0.25% (0.005%) and the highest was found in ABLS 400 ℃ at a concentration of 7% (0.965%) (Figure 2b).

Nutrient analysis of the leaves showed that there was an increase in the N, P, K, Ca, and Mg in the TWLS, PWLS, and ABLS treatments at 200 ^{0}C with a concentration of 0.25% compared with the control. The highest levels of N, P, K, Mg, and Ca were found in the ABLS at 200 °C, 0.25% concentration of 8100.0, 757.12, 3300.5, 2619.9 and 66.4 ppm, respectively. However, liquid smoke treatment at 400 ^{0}C with a concentration of 1% caused a decrease in nutrient levels compared with the control. The lowest N, P, K, and Ca were found in the ABLS at 400 °C treatment, 1% concentration of 3300, 302.09, 1686.56 and 42.27 ppm, respectively (Figure 3).

DISCUSSION

Phytotoxicity is a response in plants (germination, growth, or development) caused directly or indirectly by chemicals (Ahmed, 2018). Chemical exposure to high concentrations in plants causes phytotoxic symptoms, including wilted leaves, necrosis (browning), chlorosis (yellowing), leaf spots (yellow), brown spots, twisted leaves, and stunted or dead plants (Gadge *et al*., 2023). Liquid smoke is a natural chemical that causes phytotoxicity in plants at high concentrations. However, if the liquid smoke concentration is appropriate, it can also be used as a plant-growth agent (Wang *et al*., 2019). The dominant chemical compounds in liquid smoke were acetic acid and phenol. These compounds play a significant role in plant phytotoxicity at high concentrations (Chuah *et al*., 2016; Gadge *et al.,* 2023). This research can serve as a guideline for the amount of phenol and acetic acid that should be used in plants to avoid phytotoxicity, especially in medicinal plants (cat whiskers). Based on analytical examination, at a temperature of 400 °C and a concentration of 3%, the phenol contents of ABLS, TWLS, and PWLS were 0.034%, 0.043%, and 0.045%, respectively. The acetic acid contents were 0.418%, 0.403%, and 0.398%, respectively (Figure 2). This level causes phytotoxicity in cat whiskers plants. In addition, PWLS at 200 °C and 5% concentration caused medium phytotoxicity (score 4.0), with phenol and acetic contents of 0.026% and 0.074%, respectively. Meanwhile, at the same concentration (200 °C, 5%), the phenol content of TWLS (0.0048 %) and ABLS (0.01 %) did not cause phytotoxic symptoms (scores 0.0 of 1.0). This shows that phenol levels of <0.02% did not cause phytotoxicity in cat whiskers plants. Thus, the recommended concentration of liquid smoke for application to plants is ≤2%. These results are in line with research by Aisyah *et al*. (2018), who reported that liquid smoke from palm fronds and empty fruit bunches produced at a temperature of 400 ℃with a concentration of ≤2% did not cause phytotoxicity in banana seedlings. Chuah *et al*. (2016) proved that 200 ppm (0.02%) of pure phenolic type 2,4-Di-Tert-Butylphenol caused phytotoxic symptoms of wilting and necrosis in grassy weeds. According to Šćepanović *et al*. (2022), several types of phenol with a concentration of >0.02% are known to have an inhibitory effect on plant growth, as seen with ferulic, vanillic, p-hydroxybenzoic, p-comeric, and cinnamic acid (Anh *et al*., 2021).

Although phenol and acetic acid content at a concentration of <2% does not cause phytotoxicity in plants in soil media, it is necessary to observe phenol and acetic acid content in hydroponic applications. This research revealed that the highest phenol and acetic acid contents that were still safe in hydroponics and increased plant growth were 0.0037% and 0.023%, respectively (Figure 2), which were found in PWLS, 400 ^{0}C, 0.25% concentration. The optimal phenol and acetic acid content makes liquid smoke a growth stimulant that affects the physiology of cat whiskers plants, as evidenced by changes in root morphology, that is, an increase in root length and weight (Table 2). There was also an increase in nitrogen, phosphorus, and potassium levels in cat whiskers plants (Figure 3). This is in accordance with (Drobek *et al*., 2019), who reported that bio stimulants could promote plant growth, protect plants, and facilitate nutrient uptake.

In addition to phenol content, the composition of phenol in liquid smoke is thought to influence plant growth. In our previous research (Wibowo *et al*., 2023), we discovered that the difference in metabolites between ABLS, TWLS, and PWLS was due to the type of phenol derivative. The lignin derivative phenols syringyl and guaiacyl (hardwood lignin) dominate the TWLS. Guaycil derivative phenols (softwood lignin) dominate PWLS, whereas syringyl, guaiacyl, and hydroxyphenyl derivative phenols (grass lignin) dominate ABLS. Parvin *et al*. (2020) showed that vanilic acid (guaicyl) significantly increases the growth and salinity tolerance of tomato plants. Similarly, Ma *et al*. (2022) proved that syringic acid can increase growth and improve Pb toxicity in tomato plants.

We also discovered that the liquid smoke contains some hormone-like substances derived from lactones, specifically lactone-type 5-methyl-2(5H)-

furanone in TWLS and butyrolactone (dihydro-2(3H)-furanone) in PLWS and ABLS. The patent findings by Yonghao *et al.* (2020) claimed that gamma-butyrolactone, a lactone derivative compound, can promote plant growth at low levels (<50 µg/mL). It is believed that the combination of phenol and lactone in ABLS at 200 °C with low concentrations is more effective in promoting plant growth than other liquid smokes. Several studies have confirm that low concentrations of phenolic compounds promote plant growth, whereas high concentrations tend to inhibit plant growth (Zhai *et al.,* 2015; Zhu *et al.,* 2021).

In our hydroponic study, it was revealed that liquid smoke at 400 ^{0}C with a concentration ≥0.5% resulted in lower plant height compared to the control, indicating that phenol content at 0.5% (TWLS 0.007%, PWLS 0.0074%, and ABLS 0.0055%) can disrupt plant physiology. Moreover, higher concentration especially 2%, which has higher phenol levels (Figure 2a), led to the death of hydroponic plants. Thus, it can be proven that high concentrations of liquid smoke with high phenol content can inhibit growth and cause plant death. Several studies have shown that high phenol concentrations can be used in grass killers or herbicides (Anh *et al*., 2021; Li *et al*., 2015; Aguirre *et al*., 2020). Aguirre *et al*., (2020), studied the effects of liquid smoke as a weed herbicide, showed that using a high concentration of liquid smoke 25, 50, 75, and 100% effectively kills weeds. Therefore, stratifying pyrolysis temperatures at 200 ℃ and 400 ℃ and diluting liquid smoke at appropriate concentrations can diversify its applications. Liquid smoke at 400 ℃ is suitable for herbicides or antimicrobials, whereas liquid smoke at 200 ℃ at appropriate concentrations promotes plant growth. This study parallels the allelochemical study of plant extracts, where lower concentrations stimulated the germination and growth of plant seeds, whereas higher concentrations inhibited plant growth (Abdel-Farid *et al*., 2021; Cheng and Cheng, 2015; Grbović *et al*., 2019).

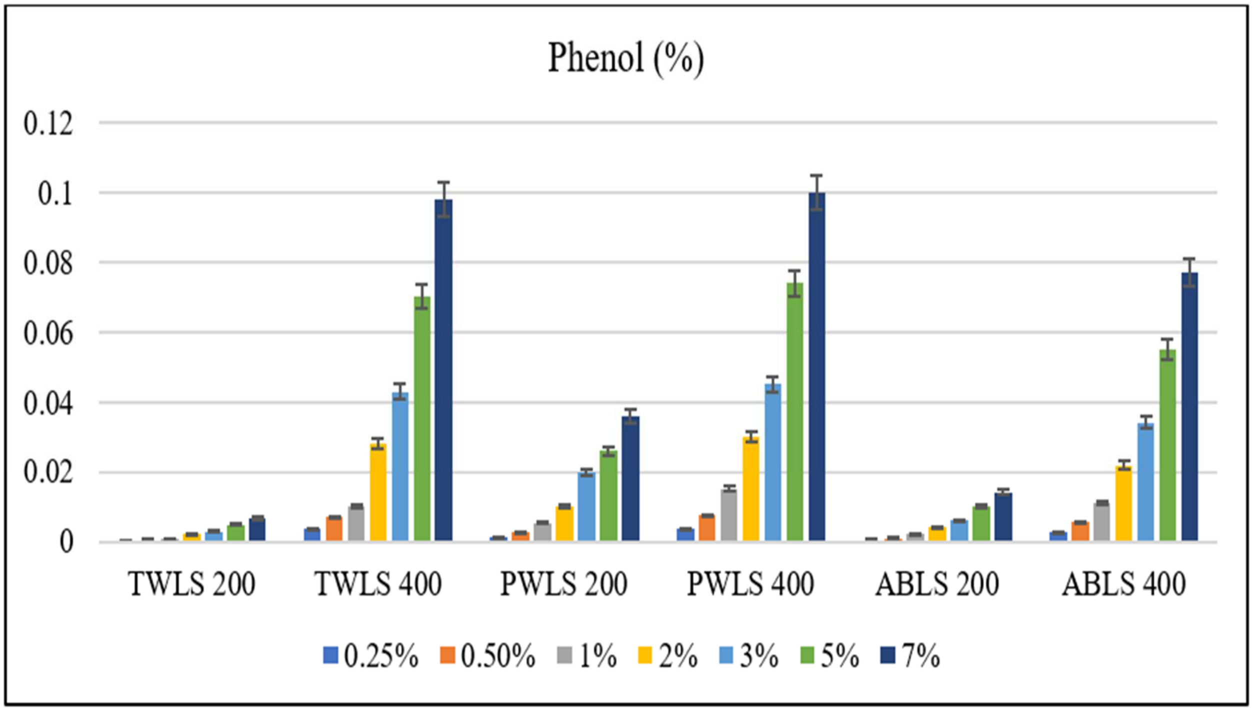
Liquid smoke not only affects growth parameters but can also stimulate secondary metabolites (sinensetin) in cat whiskers plants in hydroponic media. Sinensetin, a polymethoxylated flavonoid has been shown to have strong anticancer activity and various other pharmacological benefits including antimicrobial, anti-inflammatory, antioxidant, anti-diabetic, anti-obesity, antidementia, and has vasorelaxant effects (Hossain and Ismail, 2016; Jie *et al*., 2021). The highest sinensetin content was observed in the ABLS 200 ^{0}C treatment at a concentration of 0.25% (0.239 mg/g). The increase in sinensetin levels was caused by liquid smoke, which acts as an elicitor in cat whiskers plants. Elicitors are abiotic or biotic factors that can stimulate and enhance the biosynthesis of secondary metabolites. Elicitors play an essential role in signal transduction during plant growth and significantly affect plant bioactivity, productivity, and defense against environmental stress (Jan *et al*., 2021; Jeyasri *et al*., 2023).

This result aligns with that of Zhang *et al.* (2023), who reported that liquid smoke can act as a plant growth regulator and elicitor of plant secondary metabolites in onion and increase antioxidant and enzyme-inhibiting properties in onions. This study highlights the importance of diluting liquid smoke to avoid phytotoxicity and reveals its effectiveness concentration range for stimulating plant growth. Although promising, further research is needed to scale these findings to other hydroponic techniques and agricultural applications. This study can serve as a basis for future strategies to address the increasing problem of lignocellulosic waste. By converting this waste into charcoal and liquid smoke, it can be used in various sectors of life, particularly for plant growth. Therefore, it can replace or reduce the use of synthetic chemicals to achieve a healthy earth and sustainable natural resources.

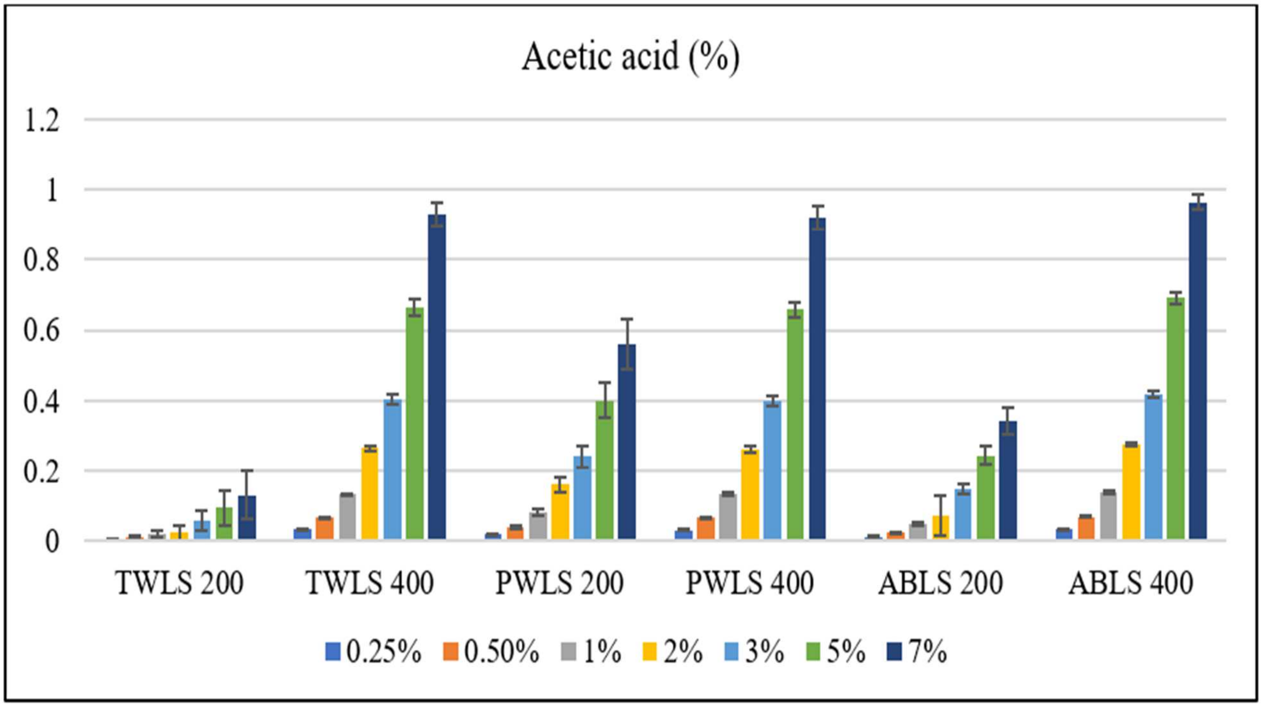
Table 2. Growth parameters and sinensetin levels of cat whiskers plants at 200 and 400 °C and different concentrations of liquid smoke.

Liquid smoke		Concentration (%)	Leaf weight (g)	Stem weight (g)	Root weight (g)	Total biomass weight (g)	Plant height (cm)	Root length (cm)	Stem diameter (mm)	Sinensetin levels (mg/g)
TWLS	200	0.25	14.43±11.63 ^{ef}	10.56±8.88 ^{f}	5.56±3.11 ^{fg}	29.03±23.86 ^{fg}	32.8±8.45 ^{f}	36.26±5.19 ^{h}	**2.1**±0.55 ^{e}	0.211±0.009
		0.5	10.45±7.35 ^{de}	7.04±5.1 ^{def}	2.04±0.89 ^{abcd}	17.93±12.6 ^{def}	30.98±9.69 ^{ef}	24.8±13.44 ^{g}	1.6±0.55 ^{cde}	-
		1	9.5± 4.54 ^{cde}	5.67±2.48 ^{cde}	2.630±1.35 ^{bcd}	16.07±7.43 ^{cde}	20.2±7.89 ^{cd}	22.82±17.86 ^{fg}	1.6±0.55 ^{cde}	-
		2	8.92±6.02 ^{bcde}	5.42± 3.53 ^{cde}	3.4±2.34 ^{cdef}	15.91±10.76 ^{cde}	27.58± 13.5 ^{def}	16.0±3.59 ^{ef}	1.44±0.81 ^{bcde}	-
	400	0.25	8.06±4.73 ^{bcde}	4.39±3.12 ^{bcde}	4.57±2.08 ^{def}	16.09±9.91 ^{cde}	23.9±11.48 ^{cdef}	7.74±2.69 ^{abcd}	1.2±0.45 ^{bcd}	0.175±0.006
		0.5	5.00±2.62 ^{abcd}	2.31±1.07 ^{abc}	2.96±2.37 ^{bcde}	9.32 ±5.35 ^{abcde}	19.44±8.31 ^{cd}	8.2±2.84 ^{abcd}	1.04±0.64 ^{bcd}	-
		1	3.01±2.9 ^{abcd}	1.82±1.72 ^{abc}	2.71±2.29 ^{bcd}	5.89±5.05 ^{abcde}	8.4±8.15 ^{ab}	0.94±0.96 ^{ab}	0.8±0.84 ^{bcd}	-
		2	* a**	* a	*a	***a**	*a	*a	*a	-
PWLS	200	0.25	7.58±3.93 ^{bcd}	4.95±2.56 ^{bcde}	3.70±2.40 ^{cdef}	14.45±8.80 ^{cde}	23.66±6.17 ^{cdef}	15.80±2.31 ^{ef}	1.6±0.55 ^{cde}	0.135±0.006
		0.5	5.19±2.42 ^{abcd}	3.20±1.43 ^{abcd}	2.22±1.47 ^{abcd}	8.81±4.47 ^{abcde}	21.98±4.0 ^{cde}	8.86±5.57 ^{bcde}	1.26±0.73 ^{bcd}	-
		1	3.16±0.55 ^{abc}	1.79±0.55 ^{abc}	0.46±0.12 ^{abcd}	4.03±1.76 ^{abc}	8.6±. 6.99 ^{ab}	2.1±1.71 ^{ab}	0.86±0.77 ^{bc}	-
		2	*a	*a	*a	***a**	*a	*a	*a	-
	400	0.25	8.82±1.51 ^{bcde}	4.81±0.26 ^{bcde}	2.64±1.26 ^{abcd}	13.26±3.53 ^{abcde}	23.0±5.86 ^{cdef}	12.1±4.47 ^{cde}	1.6±0.55 ^{cde}	0.091±0.007
		0.5	6.44±3.67 ^{abcd}	3.02±1.63 ^{abcd}	2.85±1.68 ^{bcd}	10.79±6.48 ^{abcde}	19.7±1.79 ^{cd}	4.46±1.50 ^{abc}	1.6±0.55 ^{cde}	-
		1	2.31±0.96 ^{ab}	2.25±1.30 ^{abc}	0.68±.34 ^{ab}	3.48±0.37 ^{abc}	8.6±3.78 ^{ab}	1±0.71 ^{ab}	0.72± ^{ab}	-
		2	* a	* a	*a	*a	*a	*a	*a	-
ABLS	200	0.25	**17.71±6.29** ^{f}	**8.40±2.74** ^{ef}	**6.97±2.20** ^{g}	**32.16±10.59 bcde**	**42.14 ±6.13** ^{g}	26.48±3.35 ^{g}	1.6±0.55 ^{cde}	**0.293±0.004**
		0.5	10.62±5.99 ^{de}	5.72±5.55 ^{cde}	5.46±4.98 ^{efg}	20.19±15.51 ^{ef}	28.08 ± 6.70 ^{def}	13.60±3.02 ^{de}	1.6± 0.55 ^{bcde}	-
		1	3.14±2.76 ^{abc}	2.15±0.91 ^{abc}	1.32±0.93 ^{abc}	5.40±4.83 ^{abcd}	23.3±16.95 ^{cdef}	12.8±5.08 ^{de}	1.4±0.55 ^{cde}	-
		2	* a	*a	*a	*a	* a	*a	*a	-
	400	0.25	9.76±3.32 ^{cde}	4.97±2.00 ^{bcde}	4.11±0.90 ^{def}	17.84±6.09 ^{def}	32.30±8.63 ^{ef}	11.24±4.11 ^{cde}	1.6± 0.55 ^{cde}	0.210±0.009
		0.5	7.71±0.95 ^{bcde}	4.25±1.26 ^{abcde}	2.75±0.51 ^{bcd}	13.77±2.64 ^{bcde}	14.2±3.65 ^{bc}	8.1±4.79 ^{abcd}	1.6±0.55 ^{cde}	-
		1	0.78±0.17 ^{a}	0.78±0.42 ^{ab}	0.45±0.26 ^{ab}	1.27± 0.64 ^{ab}	7.0±4.37 ^{ab}	1.6±1.56 ^{ab}	0.84±0.36 ^{bc}	-
		2	* a	*a	*a	*a	*a	*a	*a	-
Control			**7.17±3.49 bcdef**	**4.68±1.66 bcde**	**2.75±1.29 bcd**	**12.96±abcde**	**23.3± 4.3 cdef**	**20.4±9.18 ef**	1.18±0.18 bcd	**0,172±0,004**

Remark: Data are means ± SD (n = 5). The numbers with different letters in the same column indicate a significant difference at the 5 % level. TWLS: Teak Wood Liquid Smoke, PWLS: Pine Wood Liquid Smoke, ABLS: Andong Bamboo Liquid Smoke



A



B

Figure 2. Data of phenol (a) and acetic acid (b) in liquid smoke dilution (concentrations of 0.25%, 0.5%, 1%, 2%, 3%, 5% and 7%). Measurements were performed in triplicates (mean±SD) with error bars. TWLS 200: Teak Wood Liquid Smoke at 200 ℃, TWLS 400: Teak Wood Liquid Smoke at 400 ℃; PWLS 200: Pine Wood Liquid Smoke 200 ℃, PWLS 400: Pine Wood Liquid Smoke at 400 ℃, ABLS 200: Andong Bamboo Liquid Smoke at 200 ℃, ABLS 400: Andong Bamboo Liquid Smoke at 400 ℃.

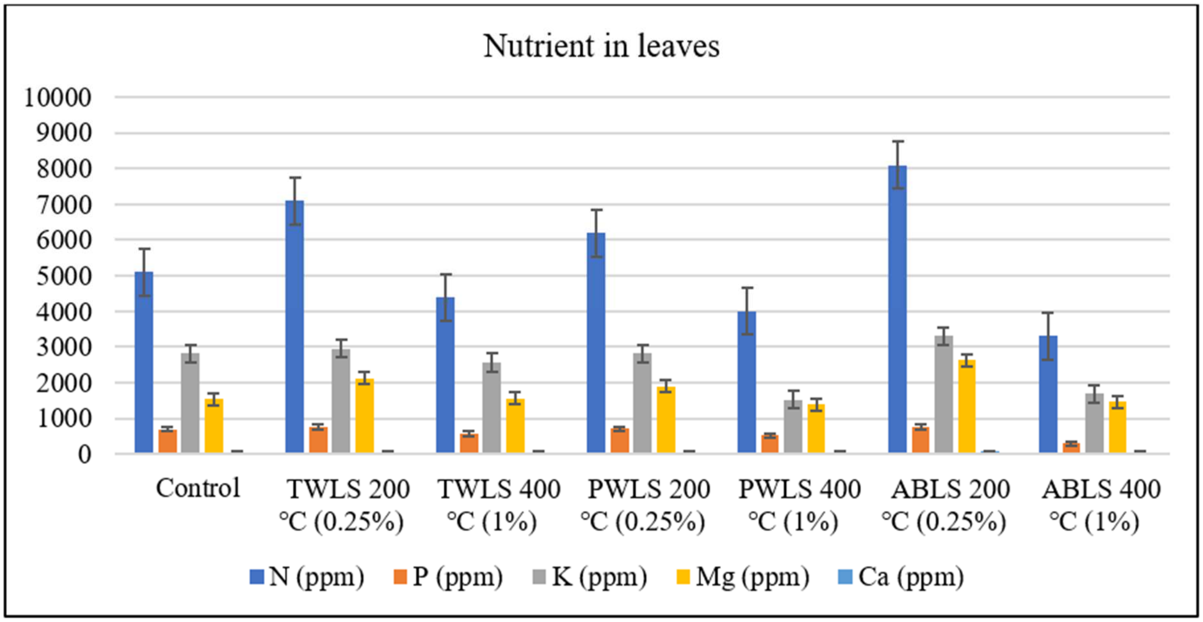


Figure 3. Nutrient data in the leaves of cat whiskers (N, P, K, Mg, Ca). Nutritional measurements for cat whiskers leaves were conducted using the highest and lowest responses of plant growth (liquid smoke at 200 °C, 0.25%, and 400 °C, 1%), along with a control. Measurements were performed in triplicates (mean±SD) with error bars. TWLS 200: Teak Wood Liquid Smoke at 200 °C, TWLS 400: Teak Wood Liquid Smoke at 400 °C; PWLS 200: Pine Wood Liquid Smoke 200 °C, PWLS 400: Pine Wood Liquid Smoke at 400 °C, ABLS 200: Andong Bamboo Liquid Smoke at 200 °C, ABLS 400: Andong Bamboo Liquid Smoke at 400 °C.

Conclusion: Liquid smoke from teak wood, pine wood, and bamboo, at 200 °C at a concentration of ≤3% is safe to for use in plants, however, liquid smoke at 400 °C and concentrations ≥3%, caused significant phytotoxicity, including leaf wilting, necrosis, chlorosis, and plant death owing to high phenol and acetic acid levels. In the hydroponic application of liquid smoke at 200 ^{0}C and a concentration of 0.25%, the growth of the cat whiskers increased significantly compared to the control. Bamboo liquid smoke treatment at 200 ^{0}C and a concentration of 0.25% generally increased growth parameters and sinensetin level compared to other treatments. This study provides knowledge to increase crop yields and cat whiskers sinensetin levels using liquid smoke with appropriate concentrations and levels of phenol or acetic acid for plant growth. However, this research must be continued with other hydroponic techniques, or applied to agricultural land, liquid smoke formulations, and applications on a larger scale.

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Author contributions: SW, WS, GP, and ENH, planned, methodology and performed data experiments. SW wrote the original draft. WS, GP, and ENH supervised the work and reviewed the manuscript. LS and SK collected samples in the field and assisted research in the laboratory. SW, WS, GP, ENH and SK interpreted the data and discussed the results. SW, LS and SK assisted in revision the manuscript.

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