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The Effect of Methyl Jasmonate on Massoia Lactone Production in Callus Culture of the Endangered Masoyi Plant (*Cryptocarya massoy* (Oken) Kosterm.)

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ABSTRACT

Cryptocarya massoy (Oken) Kosterm is a plant known for producing essential oils with many applications. However, overexploitation has threatened the sustainability of this plant. This study aims to identify the effect of adding methyl jasmonate to the culture medium on producing secondary metabolites through the callus culture of *C. massoy*. The methods in this study include callus induction, callus elicitation in liquid culture, callus extraction, analysis of secondary metabolite compounds in *C. massoy* callus culture, and data analysis. The results show a significant decrease in fresh weight of callus exposed to MeJA stress, depending on the concentration. Regarding secondary metabolites, massoia lactone, the primary compound in *C. massoy*, was not detected. It is possible that the duration of elicitation was insufficient to induce the biosynthesis and accumulation of detectable levels of massoia lactone. Secondary metabolite production often follows a specific temporal pattern, and certain compounds may require longer exposure times to reach detectable concentrations. The concentration of MeJA influenced the level of other target compound production, where at 0.5 mM MeJA increased the percentage of humulene by 24.64%. MeJA at 1.5 mM enhanced the percentage of ß-copaene (413.11%), ß-ylangene (100%). MeJA at 2 mM increased benzyl benzoate (9.61%). MeJA at 2.5 mM increased α-ylangene (31.25%) and caryophyllene (22.83%). Each MeJA concentration activated specific biosynthesis pathways, influencing the types of secondary metabolites produced. The metabolite screening revealed a predominance of terpenoids, especially sesquiterpenes, which play a role in plant defense.

1. Introduction

Masoyi (*Cryptocarya massoy* (Oken) Kosterm.) is an endemic plant of Papua (Triatmoko *et al*. 2016). This plant belongs to the Lauraceae family and can grow over 30 meters with a straight, non-twisting trunk. Its bark is grayish-green in color and has a thickness ranging from 5 to 15 mm (Rostiwati and Efendi 2013). Masoyi is an essential oil-producing plant widely used as a raw material in pharmaceuticals, perfume, flavor enhancers, cosmetics, and other industries (Sang *et al*. 2020; Zhang *et al*. 2022; Noli *et al*. 2024; Hanafi *et al*. 2025).

Masoyi is the 8th largest source of essential oil production in Indonesia. In 2016, the production of

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masoyi essential oil was 15-20 tons per year, while the global demand for masoyi essential oil reached 500,000 tons per year (Yeny and Nuroniah 2020). Meanwhile, the main supply of masoyi comes only from Papua. Indonesia's contribution to meeting this global demand is relatively low, accounting for only about 2% (Darwo and Yeny 2018). This limited supply has driven up the price of masoyi essential oil significantly, with 50% concentration priced at IDR 2.5 million per kg, 70% concentration at IDR 4 million per kg, and 90% concentration at IDR 9 million per kg (Yeny and Nuroniah 2020).

According to the IUCN Red List 2019 (Kuroh and Homot 2019), masoyi is classified as near threatened. Therefore, efforts are needed to propagate and produce its secondary metabolites through in vitro methods. Callus culture offers a promising solution to this issue. Callus culture is a technique in plant tissue culture where plant tissues (usually parenchyma cells) are induced to form an undifferentiated mass of cells. Callus formation occurs in response to wounding or applying growth regulators, such as auxins and cytokinins, in an appropriate nutrient medium. Callus culture plays a crucial role in various aspects of plant biotechnology, such as somaclonal variation, somatic embryogenesis, crop improvement, disease elimination, and the production of secondary metabolites (Hussain *et al*. 2012; Noli *et al*. 2025). A promising approach to enhance the production of secondary metabolites in plants is by utilizing callus culture combined with the addition of elicitors (Ali *et al*. 2018; Ali *et al*. 2019; Mahood *et al*. 2022; Bano *et al*. 2022).

Elicitors are substances or agents used to stimulate or enhance the production of secondary metabolites in plants grown in vitro. One of the most commonly used elicitors is methyl jasmonate (MeJA). MeJA is a hormonal elicitor that activates enzymes and induces defense-related gene expression (Murthy *et al*. 2014). MeJA has been successfully used to enhance the production of secondary metabolites in plants. Rahmati *et al*. (2023) reported that adding 50 µM MeJA to *Carum carvi* L. callus culture significantly increased carvone and limonene levels. Abd El-Kader *et al*. (2019) also reported that a similar treatment in *Cinnamomum camphora* callus culture successfully increased d-limonene levels. Additionally, MeJA is also used to enhance the production of other metabolites, such as secoiridoid glycosides (Mahendran & Rahman 2024), cycloartane saponins (Enchev *et al*. 2024), and prenylated stilbenoids (Chayjarung *et al*. 2022). Secondary metabolites, particularly massoia lactone is typically synthesized and released by plants in response to tissue damage, particularly that caused by herbivore attacks. These compounds play an important role in indirect defense mechanisms, such as attracting predatory or parasitoid arthropods that target the herbivores, thereby helping to reduce further damage. In this context, MeJA functions as an elicitor that mimics biotic stress signals in a non-invasive manner, enabling the induction of plant defense pathways without physical damage (Martin *et al*. 2003). MeJA binds to specific receptors in plant cells, thereby activating intracellular signaling cascades that lead to the generation of reactive oxygen species (ROS). These ROS act as secondary messengers that trigger the transcriptional activation of genes involved in the biosynthesis of massoia lactone. As a result, the expression of key biosynthetic enzymes is upregulated, ultimately leading to an enhanced accumulation of massoia lactone in plant tissues (Nabi *et al*. 2021).

So far, there is no available information on the in vitro elicitation of *C. massoy*. Therefore, this study aims to identify the effect of adding methyl jasmonate to the tissue culture medium on massoia lactone production through the callus culture of this plant.

2. Materials and Methods

2.1. Plant Materials and Sterilization

C. massoy nodal explants were obtained from PT. Mitra Ayu Adi Pratama, Padang, Indonesia. The explants were excised from healthy donor plants and carefully transported under sterile conditions to the laboratory for further in vitro culture. The explants were cleaned by gently scrubbing to remove external contaminants attached to the nodes. The explants were then sterilized using Tween 20 for 10 minutes and then using the fungicide Dithane for 10 minutes. Sterilization continued with 4 mg/L bactericide Agrep, 10% sodium hypochlorite, and 5% sodium hypochlorite, for 10 minutes, respectively. Under aseptic conditions, the explants were sterilized with four tetracycline bactericide capsules in 500 ml of distilled water, one ketoconazole fungicide capsule in 100 mL of 96% alcohol, and 0.1% $HgCl_2$ for 10 minutes, respectively. The explants were then sterilized with vitamin C for 10 minutes and rinsed with 70% alcohol for 3 minutes. A single rinse with distilled water followed each sterilization step.

2.2. Callus Induction

The basal medium used was 1/2 MS (Murashige and Skoog 1962) supplemented with 30 g/L sucrose, 5 mg/L Benzyl Amino Purine (BAP), solidified with 7 g/L agar, and the pH adjusted to 5.8. All sterilized explants were cut into 2 cm, planted on the agar medium, and incubated for 2 months under controlled conditions at 25°C with a constant 24-hour photoperiod.

2.3. Callus Propagation and Elicitation

The callus obtained from the callus induction stage was weighed at 13 mg and then transferred to liquid media containing 1/2 MS (Murashige and Skoog 1962), 30 g/L sucrose, pH 5.8, and 5 mg/L Benzyl Amino Purine (BAP), and incubated on a shaker for 5 days at 50 rpm. After 5 days, the elicitor MeJA, obtained from Aldrich Chemical Co. USA, was added to the liquid medium according to treatment 0 (control), 0.5 mM, 1 mM, 1.5 mM, 2 mM, and 2.5 mM, with three replications. MeJA was added to the media using a syringe and 0.22 µm filter. The callus was then incubated on the shaker at 50 rpm for 3 days at 25°C with 24-hour photoperiod.

2.4. Callus Extraction

The callus obtained from the suspension culture was rinsed using distilled water and then ground into paste-like consistency for subsequent extraction.. The sample was placed in a 15 ml tube and extracted with 0.5 ml of ethanol, followed by vortexing until homogeneous, incubation for 5 minutes, and centrifugation at 3000 rpm for 1 minute. The ethanol phase was transferred to a new conical tube, and the residue was re-extracted by adding 0.5 ml of ethanol. The extracted sample was transferred to GC-MS vial using a 0.20 µm filter.

2.5. Phytochemical Analysis

The samples were analyzed at the Integrated Research and Testing Laboratory of Gadjah Mada University (LPPT UGM) for analysis using GC-MS (Thermo ScientificTM TRACE 1310 GC and ThermoScientificTM ISQ LT Single Quadrupole Mass Spectrometer). The GC-MS settings were configured in accordance with the standard operating procedures established by the LPPT UGM. The stationary phase used was an HP-5MS UI column (30 m × 0.25 mm, 0.25 µm film thickness), and the mobile phase was UHP helium (He) at a pressure of 23.10 psi. The settings were as follows: split flow: 10 ml/min, split ratio: 10, front inlet flow: 1.00 ml/min, MS transfer line temperature: 280°C, ion source temperature: 280°C, mass range (amu): 40–500, purge flow: 3 ml/min, and runtime: 39 minutes. The injector temperature was 250°C. The oven temperature program was set as follows: initial time 2.0 minutes, initial temperature 0°C, final temperature 280°C, program rate from 2 to 10 minutes (4°C/min), 11–35 minutes, and final time 39 minutes. The detector was configured based on the method reported by Rali *et al*. (2007), with the following settings: ionization 70 eV, transfer temperature of 310 °C, quadrupole temperature 150 °C, source temperature 230 °C, and mass scan range of 35–550 m/z. Chromatogram software (Chromeleon 7) and the NIST 2014 library database were used. The results obtained were in the form of chromatogram with several peaks based on retention time. GC-MS analysis provided data on the peaks, retention times, relative percentage (% area), and the names of the identified compounds.

2.6. Data Analysis and Statistics

Data were analyzed both qualitatively and quantitatively. The qualitative analysis included observations of texture, color, callus survival percentage, and phytochemical analysis of callus. Quantitative analysis involved measurements of fresh weight. Quantitative data were presented as mean ± standard deviation. Data analysis was performed using IBM SPSS software. Statistical analysis was conducted using ANOVA, and if significant differences were observed, Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) was applied. The GC-MS results were then analyzed using the Venn diagram on the Jvenn website (https://www.bioinformatics.com.cn/static/others/jvenn_en/index.html).

3. Results

3.1. Callus Growth with MeJA Treatment

The callus in each treatment had a compact texture, indicating that MeJA treatment has no negative effect on callus texture. Meanwhile, the callus exhibited white and brown colors (Table 1).

The explants were initiated on media containing 5 ppm BAP. After 2 months, the explants showed swelling, but only some developed callus. Callus growth was not optimal, as most explants exhibited swelling without callus formation. Following the callus initiation, MeJA

was added to the liquid media. Green explants were only observed in the 0 mM MeJA treatment (control), while other treatments exhibited browning (Figure 1).

The highest fresh weight of callus was observed in the 0 mM MeJA treatment (15.09 mg), while the lowest was in the 2.5 mM MeJA treatment (12.14 mg) (Figure 2). This indicates that higher MeJA concentrations result in decreased callus biomass. However, the results of this study did not show significant differences among the treatments ($p<0.05$).

Table 1. Texture, color, and percentage of callus survival after three days of MeJA elicitation

Treatment	Texture	Color	Percentage of callus survival
MeJA 0 mM	Compact	7.5 Y 8.5/4	100
MeJA 0.5 mM	Compact	5Y 8/4	100
MeJA 1 mM	Compact	10 YR 6/6	100
MeJA 1.5 mM	Compact	10 YR 6/6	100
MeJA 2 mM	Compact	2.5 YR 3/2	100
MeJA 2.5 mM	Compact	2.5 YR 3/2	100

Observations were conducted on the final day of elicitation (day 3). Color references were based on the munsell book of color.

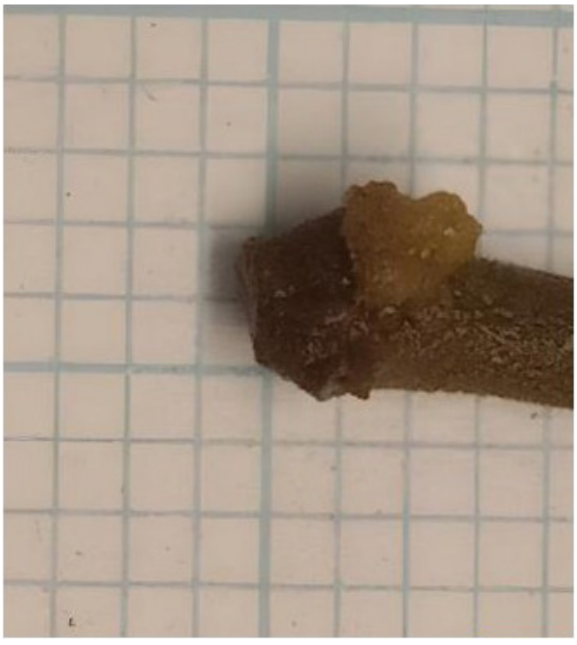

Figure 1. Callus of *C. massoy* derived from nodal explants (block size: 1 mm × 1 mm). Observations were conducted at three days after elicitation

3.2. Secondary Metabolite Profile of Masoyi Callus Determined via GC-MS

Based on GC-MS analysis, massoia lactone was not detected in any of the samples elicited for three days with MeJA concentrations ranging from 0 to 2.5 mM. This may be attributed to the relatively young age of the callus (one month) and the short duration of elicitor treatment (three days). Hence, MeJA treatment concentrations may not have influenced the metabolic signaling pathways involved in massoia lactone biosynthesis. Nevertheless, MeJA was able to induce the biosynthesis of other targeted secondary metabolites with known bioactive properties (caryophyllene, α-ylangene, ß-ylangene, ß-copaene, humulene, benzyl benzoate). MeJA treatment increased the percentage of other targeted compounds in masoyi callus compared to the control. Specifically, 0.5 mM MeJA increased the percentage of humulene by 24.64%. MeJA at 1.5 mM enhanced the percentage of ß-copaene (413.11%), ß-ylangene (100%). MeJA at 2 mM increased benzyl benzoate (9.61%). MeJA at 2.5 mM increased α-ylangene (31.25%) and caryophyllene (22.83%). These findings suggest that each MeJA treatment concentration may influence the accumulation of different secondary metabolites. Based on Table 2, sesquiterpenes (58.21%) were identified as the most dominant group of compounds in *C. massoy* callus cultures, followed by esters (31.40%) and diterpenoids (0.63%).

The Venn diagram analysis shows the distribution of compounds among different treatments. Based on Figure 3, Nine chemical compounds were identified across the control and MeJA treatments at concentrations of 0.5, 1, 1.5, 2, and 2.5 mM, consisting of seven sesquiterpenoids and two esters. One new compound, (S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene, was detected in the 1 mM MeJA treatment. Two new compounds, (1S,4aR,8aS)-1- Isopropyl-7- methyl-4-methylene-1,2,3,4,4a,5,6,8a- octahydronaphthalene and cis-α-Bisabolene were identified in the 1.5 mM treatment. Another compound (α-aconero) l was observed in the 0.5, 1, and 1.5 mM treatments. Furthermore, (1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol was detected in the 1.5 and 2.5 mM treatments. Hexanoic acid, 2-phenylethyl ester was identified in the 0.5 and 2.5 mM MeJA treatments.

4. Discussion

The response of *C. massoy* callus to MeJA was a reduction in growth, a common drawback of using abiotic elicitors. In this study, changes in the fresh weight of the

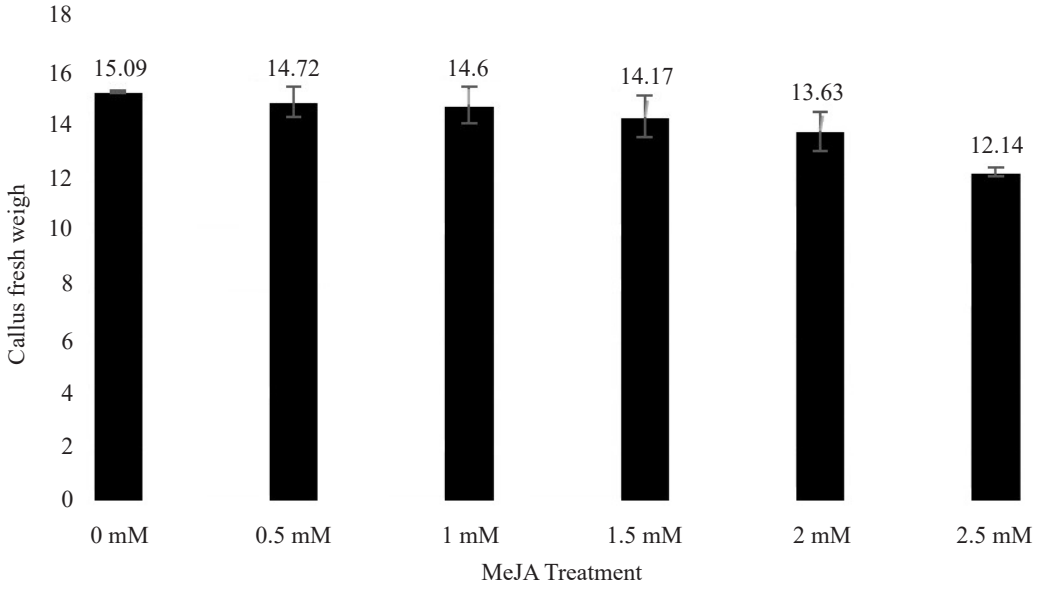

Figure 2. Effect of treatments with varied MeJA concentrations (0, 0.5, 1, 1.5, 2, and 2.5 mM) on masoyi callus weight. Data represent mean (with 3 replications) ± standard deviation. Observations were conducted at three days after elicitation

Table 2. Relative percentage (% area) of compounds present in masoyi callus elicited with MeJA

Group	RT (min)	Compound name	Concentration (% area)					
			MeJA 0 mM	MeJA 0.5 mM	MeJA 1 mM	MeJA 1.5 mM	MeJA 2 mM	MeJA 2.5 mM
Sesquiterpen	6.82	α-ylangene	0.80±0.22	0.88±0.09	0.56±0.33	0.70±0.24	0.77±0.08	1.05±0.53
	8.76	ß-copaene	0.61± 0.42	0.82±0.32	0.64±0.27	3.13±2.88	1.12±0.68	1.39±0.96
	7.45	Caryophyllene	27.01±4.03	33.02±0.24	29.36±5.17	23.57±7.21	27.08±4.31	33.17±8.03
	7.54	ß-ylangene	0.69±0.26	0.96±0.32	0.99±0.15	1.38±0.77	1.16±0.22	0.94±0.23
	7.91	Humulene	2.11±0.60	2.63±0.52	1.84±0.35	1.26±1.10	0.76±1.07	2.18±0.64
	8.67	7-epi-cis-sesquisabinene hydrate	0.88±0.92	0.63±0.18	1.66±0.30	0.29±0.26	1.43±0.68	0.96±1.67
	10.93	trans-Z-α-Bisabolene epoxide	0.23±0.20	-	-	-	0.17±0.24	-
	8.27	Germacrene D	11.09±3.49	9.04±0.13	10.86±2.25	4.39±7.60	-	3.93±6.81
	8.51	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	2.71±2.42	2.33±3.30	3.39±2.94	3.43± 2.09	2.68±3.79	2.04±3.53
	8.67	6-epi-shyobunol	0.97±1.39	-	-	1.73±2.99	-	-
	8.75	α-aconerol	-	0.53±0.74	0.35±0.61	0.12±0.21	-	-
	8.68	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylene-cyclodeca-1,6-diene	-	-	0.41±0.71	-	-	-
	8.17	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4-methylene-1,2,3,4,4a,5,6,8a-octahydronaphthalene	-	-	-	0.71±0.62	-	-
	10.95	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	-	-	-	0.15±0.27	-	0.15±0.27
	7.92	cis-α-Bisabolene	-	-	-	0.34±0.58	-	-
	8.26	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7] decane-rel-	8.72±7.57	4.58±6.47	-	7.20±6.62	8.83±1.11	5.34±4.63
	8.59	Nerolidyl acetate	2.39±3.49	1.36±0.47	0.16±0.28	-	2.23±3.15	0.36±0.62
Total			58.21	56.78	50.22	48.40	46.23	51.51

Table 2. Continued

Group	RT (min)	Compound name	Concentration (% area)					
			MeJA 0 mM	MeJA 0.5 mM	MeJA 1 mM	MeJA 1.5 mM	MeJA 2 mM	MeJA 2.5 mM
Diterpenoid	27.17	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	0.63±1.10	0.39±0.13	0.55±0.96	0.31±0.03	-	0.13±0.22
Total			0.63	0.39	0.55	0.31	-	0.13
Ester	14.48	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans-	0.61±0.48	-	-	-	0.17±0.23	-
Ester	13.44	Benzoic acid, 2-hydroxy-, phenylmethyl ester	8.92±1.76	9.85±4.93	11.96±4.18	12.12±1.64	11.62±0.66	11.09±2.26
Ester	11.99	Benzyl Benzoate	17.89±3.09	17.65±0.87	16.40±2.27	17.51±1.06	19.61±0.84	13.10±4.84
Ester	13.19	Hexanoic acid, 2-phenyl-ethyl ester	-	0.16±0.22	-	-	-	0.17±0.29
Total			27.42 86.26	27.66 84.83	28.30 79.07	29.63 78.34	31.40 77.63	24.36 76.00

Relative percentages (% area) data represent mean (with 3 replications) ± standard deviation. Observations were conducted at three days after elicitation. RT: Retention Time

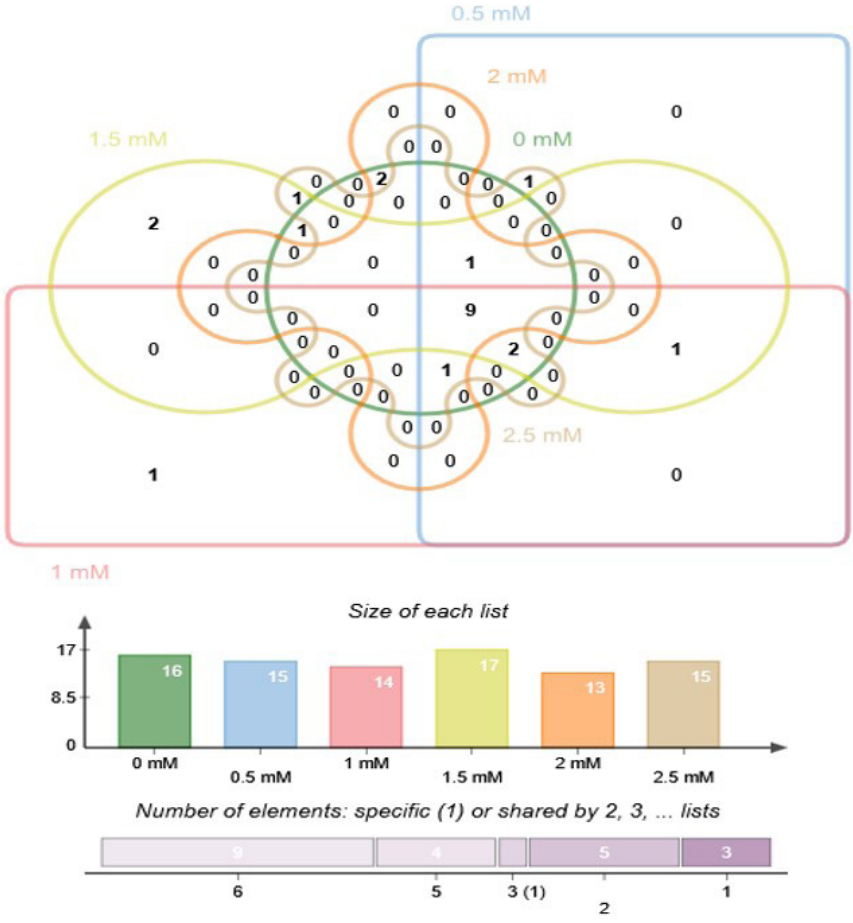

Figure 3. Venn diagram of compound distribution among MeJA treatments (0, 0.5, 1, 1.5, 2, and 2.5 mM)

callus were observed three days after the application of the elicitor. The results showed a significant decrease in fresh weight in callus exposed to MeJA stress, depending on the concentrations applied. D'Alessandro *et al*. (2022) reported that MeJA treatment reduced the fresh weight of *Cynara cardunculus* L. var altilis callus cultures. Similar effects were observed in *Nardostachys jatamansi* callus (Rawat *et al*. 2020), *Allium jesdianum* (Yazdanian *et al*. 2022), and *Aquilaria malaccensis* (Faizal *et al*. 2022). MeJA inhibits biomass growth by disrupting cortical microtubules (Koda *et al*. 1997). When plants are under stress, energy usually used for development is redirected to defense mechanisms. Furthermore, osmotic stress caused by the elicitor can interfere with the cell cycle, leading to inhibited cell division (Skirycz *et al*. 2011).

Callus color showed that the callus in the control treatment (without MeJA elicitation) had a pale yellow color. Meanwhile, the callus treated with MeJA underwent color change to darker shade. This change tended to occur more rapidly with increasing concentrations of the MeJA elicitor (Table 1). The browning of the callus indicates that it is undergoing stress, which may be induced by various factors, including the application of an elicitor. This phenomenon is closely associated with oxidation, ethylene, and programmed cell death (Chaudhary *et al*. 2015; Zhang *et al*. 2020).

The main compound in *C. massoy* is massoia lactone. Based on GC-MS analysis, massoia lactone was not detected in any of the samples elicited for three days with MeJA concentrations ranging from 0 to 2.5 mM. According to Halder *et al*. (2019), this absence may be attributed to several factors. First, it is possible that the duration of elicitation was insufficient to induce the biosynthesis and accumulation of detectable levels of massoia lactone. Secondary metabolite production often follows a specific temporal pattern, and certain compounds may require longer exposure times to reach detectable concentrations. Second, the applied concentrations of MeJA may not have been optimal for activating the biosynthetic pathway responsible for massoia lactone production. Each secondary metabolite has a distinct elicitation threshold, and suboptimal doses may fail to trigger its synthesis. Singh & Dwivedi (2018) and Petrova *et al*. (2024) stated that the effectiveness of elicitation is determined by several factors, including elicitor concentration, duration of exposure, selectivity, age of culture, growth regulation, cell line, quality of cell wall materials, and nutrient composition. Nevertheless, in this study, MeJA was able to induce the biosynthesis of other targeted secondary metabolites with known bioactive properties (caryophyllene, α-ylangene, ß-ylangene, ß-copaene, humulene, benzyl benzoate).

In this study, the concentration of MeJA influenced the level of target compound production, where at 0.5 mM MeJA increased the percentage of humulene by 24.64%. MeJA at 1.5 mM enhanced the percentage of ß-copaene (413.11%), ß-ylangene (100%). MeJA at 2 mM increased benzyl benzoate (9.61%). MeJA at 2.5 mM increased α-ylangene (31.25%) and caryophyllene (22.83%). Under stress conditions induced by MeJA at specific concentrations, a number of genes are activated and play roles in defense mechanisms as well as the biosynthesis of certain secondary metabolites. As reported by Dai *et al.* (2024) and Yang *et al*. (2025), under stress conditions, the synthesis of caryophyllene is regulated through specific genetic pathways involving genes such as TsERF66 and TsTPS18, which encode caryophyllene synthase enzymes, this leads to an increase in caryophyllene content. Similarly, Cheng *et al*. (2007) found that MeJA-induced stress at 250 µM at a concentration of 250 µM activated the OsTPS3 gene leading to increased caryophyllene accumulation in *Oryza sativa* L. Kumeta & Ito (2016) also reported that exposure to 0.1 mM MeJA elicitor induced the expression of AcHS1-3 genes in *Aquilaria crassna*, which subsequently activated humulene synthase enzymes, resulting in the production of humulene. A comparable mechanism was observed by Ran *et al*. (2023) demonstrated that MeJA-induced stress at 1 mM increased the expression of the AsHS1 gene in *Aquilaria sinensis*, along with enhanced activity of humulene synthase, leading to a higher accumulation of humulene at that concentration. Alwakil *et al*. (2022), where applying MeJA 400 µM + SA 400 µM increased zerumbone content, and MeJA 400 µM + SA 600 µM increased humulene content. Chen *et al*. (2021) reported that MeJA 200 µM increased germacrene D content. Several other genes are also involved in stress responses, particularly in response to MeJA elicitation. For example, the BAMT gene is responsible for benzyl benzoate production in Camellia sinensis (Gao *et al*. 2023), while the Copu2 gene activates β-copaene synthase in *Coniophora puteana* (Mischko *et al*. 2018).

The metabolite screening results showed that most of the *C. massoy* callus extracts were dominated by terpenoid compounds, particularly sesquiterpenes. Shi *et al*. (2015) stated that various plant species produce volatile and non-volatile compounds. Volatile compounds, which include terpenoids (hemiterpenoids [C5], monoterpenoids [C10], sesquiterpenoids [C15], and some diterpenoids [C20]), are crucial for plant-insect

interactions and are involved in general defense responses or stress induced by exogenous elicitors. According to Ali *et al*. (2019), Methyl jasmonate (MeJA) is known to induce enzymes involved in plant stress responses, such as Superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). These enzymes play a role in defense against accumulating ROS. Ho *et al*. (2020) also explained that MeJA can activate ROS as an initial signal, activating defense mechanisms in plant cell cultures and organs to combat various environmental stress conditions. It leads to decrease in callus growth and increase in secondary matabolite production.

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