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Anne Carolina[1], Rita K. Sari[1], Deded S. Nawawi[1], Effendi T. Bahtiar[1] and Dai Kusumoto[2]

Mechanical-chemical induction of balsam from *Liquidambar excelsa* trees

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Highlights

- The combination of mechanical and chemical induction on Rasamala branches offers an easy and efficient method for balsam exudation.
- Balsam exudation is chemically stimulated by methyl jasmonate and ethephon.
- Stimulant concentration increased the amount of balsam exuded in a dose-dependent manner.

Abstract

Rasamala (*Liquidambar excelsa* (Noronha) Oken) is an endemic plant in Indonesia. Apart from its use as wood, Rasamala also produces an exudate, known as balsam. Rasamala balsam has the potential to be a substitute for other true balsams derived from Altingiaceae, namely Storax. However, local communities have not used Rasamala balsam to its full potential owing to a lack of knowledge about the tapping method and processing. Therefore, an easy and efficient induction method for plant exudates is required to boost productivity. The use of exogenous hormones as stimulants and less damaging tapping techniques for plant stems requires further investigation. In this study, mechanical and chemical inductions were conducted using 0.1%, 1%, 2%, 5%, and 10% (w/w) methyl jasmonate and ethephon as stimuli. These chemical compounds were applied to young twigs without incision (TW), by incision (TI), to branches perforated with an electric bore (BB), and by incision (BI). After exogenous application for 21 days, Rasamala balsam exuded in all induction techniques, except for the TW treatment. BI treatment showed the highest effective induction, as indicated by the highest balsam exudation. Furthermore, methyl jasmonate was a better chemical stimulant than ethephon. In addition, the induced balsam Rasamala exudate showed a physical characteristic of a clear, thick, sticky colorless to white liquid with a distinctive balsamic odor.

Keywords *Liquidambar excelsa*; balsam; ethylene; methyl jasmonate; sustainable tapping
Addresses [1]Department of Forest Products, Faculty of Forestry and Environment, IPB University, Jl. Lingkar Kampus IPB Dramaga, Bogor, 16680, Indonesia; [2]The University of Tokyo Chiba Forest, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 770 Amatsu, Kamogawa, Chiba 299-5503, Japan
E-mail a_caroline@apps.ipb.ac.id
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1 Introduction

Indonesia, a mega-biodiversity country has a variety of resources (LIPI 2014), including Rasamala (*Liquidambar excelsa* (Noronha) Oken), a balsam producer that is still underutilized (Muhaimin and Nurlaeni 2018). Rasamala is a forestry commodity with great economic value because of its wood strength. Furthermore, in response to wounding and fungal attacks, Rasamala secretes balsam. This fragrant exudate can be used in fragrances or as a tonic by the local communities (Purnawan 2006; Jumali 2006).

Balsam is defined as a plant exudate containing cinnamic and benzoic acids along with their derivatives (Aguiar et al. 2022). Balsam derived from *L. orientalis* Mill., Altingiaceae is regarded as true balsam and named "Styrax liquidus" (Custódio and Veiga-Junior 2012), storax, or Turkish sweetgum. In addition, storax derived from *L. styraciflua* L. is called American storax (Lingbeck et al. 2015). Genus Liquidambar is recognized for its balsamic exudations (Honda et al. 1996) and is valuable globally for its lumber and fragrant resin, as well as locally for the roots and bark used in traditional Chinese medicine (Ickert-Bond et al. 2007). Meanwhile, balsamic resin from Rasamala called "getah malai" is used as incense (Soerianegara and Lemmens 1993). Balsam is widely used in the perfume industry as a flavor fixative, used to flavor tobacco and soap, among other cosmetic applications (Hafizoglu et al. 1996).

Stimulation of plant exudate formation has traditionally been carried out mechanically by slashing the bark of the trunks of trees with wide and irregular incision sizes in one tree (Boer and Ella 2001). In addition, increasing the productivity of plant exudates can also be stimulated chemically, including the exogenous application of sulfuric acid, 2-chloroethylphosphonic acid (CEPA), which is the precursor of the plant hormone ethylene (Rodrigues-Corrêa and Fett-Neto 2012). Furthermore, salicylic acid has recently been discovered as an active ingredient in pine resin-tapping stimulant pastes (Rodrigues and Fett-Neto 2009). In contrast, Junkes et al. (2019) observed a considerable increase in pine resin production with a methyl jasmonate stimulant.

Conventional stimulants based on strong acids have long been used by Perhutani, a state-owned enterprise and one of the manufacturers of pine resin in Indonesia, to increase pine resin production (Sukadaryati 2014). Moreover, induction using ethylene, salicylic acid, and methyl jasmonate has been previously studied in conifers (Hudgins et al. 2004; Liu et al. 2022). However, ethylene and methyl jasmonate as plant hormones stimulate physiological activities in trees and promote their own functions, without critical disorder, resulting in more beneficial use than sulfuric acid. Some previous studies have demonstrated the induction of gum duct formation and gum exudation in broad-leaved trees using methyl jasmonate and ethylene as stimulants (Boothby 1983; Morrison et al. 1987; Saniewski et al. 2004; Matsumoto et al. 2009). However, experiments involving the chemical induction of Rasamala balsam are limited. In this study, we investigated mechanical-chemical induction techniques to determine the most effective and sustainable method for enhancing Rasamala balsam production, by analyzing balsam production and its physical characteristics.

2 Material and methods

2.1 Tree material, methyl jasmonate and ethephon stimulant

In this study, we used 5 trees of 5-year-old Rasamala (*Liquidambar excelsa*) with a diameter at breast height (DBH) of approximately 12–14 cm and a height of 7–8 m planted in the garden office of Graha Waskita in Bogor, West Java, Indonesia. The twigs and branches were arranged in ascending order to form irregularly globular crowns facing the sun to the east. Trees were identified by the Indonesian Ministry of Environment and Forestry's Center for Standardization of Sustainable Forest Management Instruments (Bogor, Indonesia). Rasamala belongs to the genus *Liquidambar* in the Altingiaceae, closely connected to the Hamamelidaceae (Ickert-Bond and Wen 2013). It grows widely in China, India, Myanmar, Malaysia, and Indonesia (Huang et al. 2021). Rasamala is an evergreen, monoecious tree, has a straight bole with branches rising above the ground, that can grow to 60 m in height and 1.5 m in diameter (Van Steenis et al. 2006).

Mixtures of Tween-80 (Merck, Darmstadt, Germany) with 0.1%, 1%, 2%, 5%, and 10% (w/w) methyl jasmonate (Phytotechlab, Kansas, USA) and ethephon (Bayer, Jakarta, Indonesia) were prepared.

2.2 Induction of Rasamala balsam

Each Rasamala tree received four induction treatments (Fig. 1). Ethephon and methyl jasmonate at 0.1, 1, 2, 5, and 10% (w/w) concentrations were applied about of 0.5 ml using spuit syringe, as follows:

(a) the twigs with a diameter about of 1 cm without incision (TW),
(b) the incised twigs, with a diameter about of 2 cm, peeled off to expose the sapwood surface over an area of approximately 2 cm^2 (2 cm × 1 cm) in the longitudinal direction (TI),
(c) the branches with a diameter of about 4 cm, incised area of approximately 2 cm^2 (2 cm × 1 cm) in the longitudinal direction (BI),
(d) the drilled branches with a diameter of about 4 cm, holed using an electric drill (chuck size of 0.5 cm) with a depth of approximately 0.5 cm (BD).

The treated sites were wrapped in clear plastic and sealed to collect exudates. Mechanical and chemical induction were applied only once, but the observation continued for up to 21 days. The experiments were conducted thrice from May−August 2022. Exudates from each treatment were collected in plastic bottles.

2.3 Physical observation of Rasamala balsam exudates

The physical properties of the induced balsam, including color, odor, dirt content, ash content, and melting point, were determined using the AOAC standard method (AOAC 2008). The total ash content was determined by measuring the total amount of residual material remaining after ignition.

2.4 Data analysis

The effect of induction techniques, chemical stimulants, and stimulant concentrations were analysed statistically with three-factorial ANOVA using IBM SPSS 25.0 software. The mean Rasamala balsam was defined by three experiments from May−August 2022.

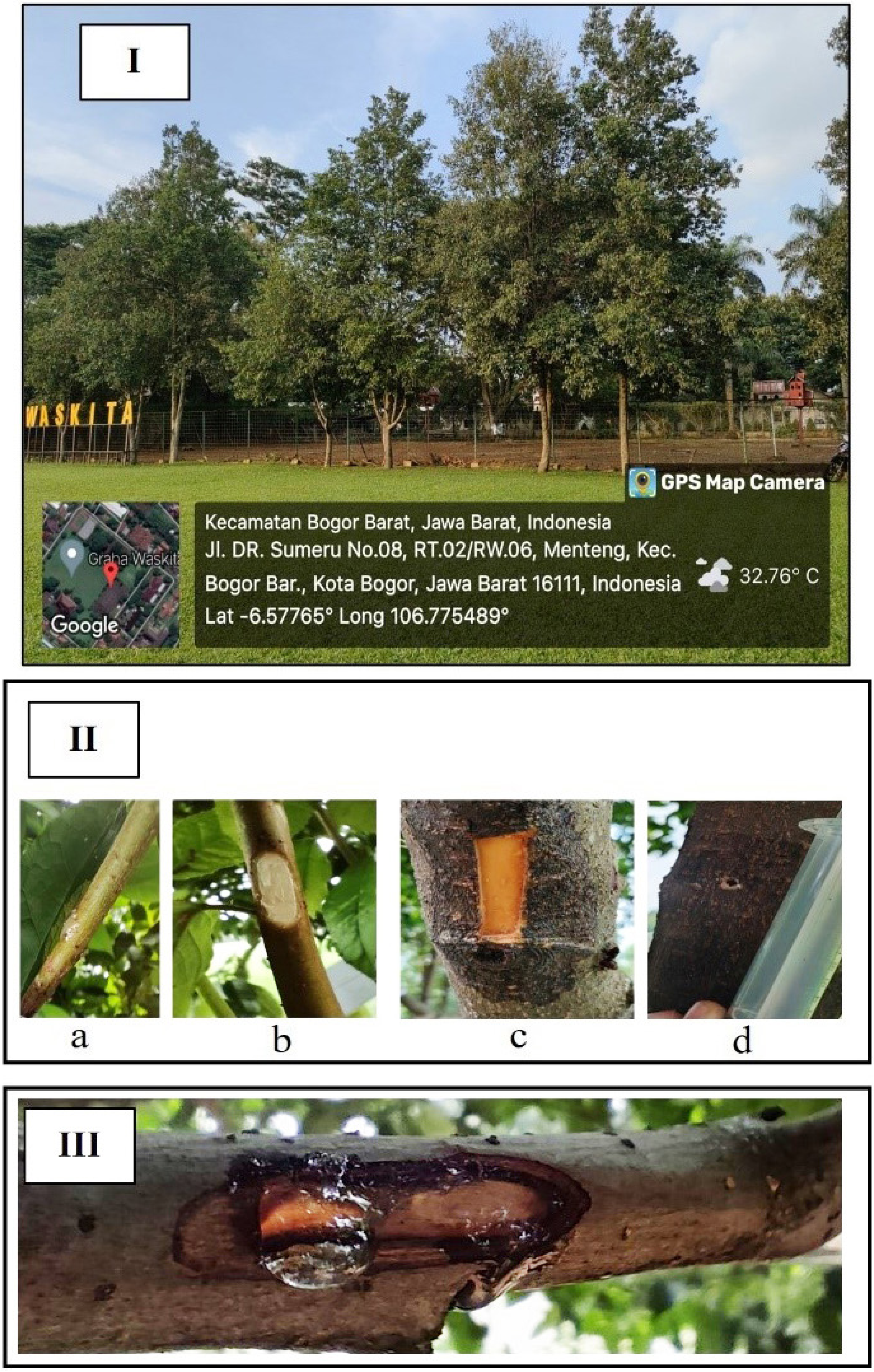

Fig. 1. (I) Rasamala trees planted in Graha Waskita, Bogor, West Java, Indonesia, (II) The treatments of methyl jasmonate and ethephon applied to (a) twig without incision (TW), (b) incised twig (TI), (c) incised branch (BI), (d) drilled branch (BD), (III) Rasamala balsam exuded from the incised twig.

3 Results

3.1 Rasamala balsam yield and different induction techniques

This study revealed that the induction techniques had a significant effect on balsam yield. During the 21 days of observation, mechanical-chemical induction by making incisions on the Rasamala twigs (TI), branches (BI), and drilled branches (BD) produced exudates in all replications. In contrast, twigs without incision (TW) did not induce Rasamala balsam formation at all. Therefore, three-factorial ANOVA was performed to determine the effect of chemicals (ethephon and methyl jasmonate), the concentration of chemicals (0.1%, 1%, 2%, 5%, and 10% (w/w)), and mechanicals (twigs incision, branches incision, and drilling branches) treatments on the resulting balsam exudates. Table 1 shows the significant differences in the interactions of the induction techniques with Rasamala balsam exudation. The treated branches produced more balsams than treated twigs. In addition, the incision technique induced more balsam exudation than the drill-hole technique.

The results confirmed that the exudation of Rasamala balsam could be expedited both by methyl jasmonate and ethephon. However, this study showed that methyl jasmonate as a stimulus is more effective than ethephon in inducing balsam production at the same concentration. This study indicated that 10% methyl jasmonate resulted in the best balsam production in Rasamala.

3.2 Physical observation of the induced balsam of Rasamala

The Rasamala balsam exudate is colorless when it is first exuded from the tree. After solidification, the color changed from colorless to white-pale yellow depending on the impurities present in the balsam. It is a viscous, sticky, and water-insoluble liquid with a strong sweet balsam odor. The results showed that the total ash content of Rasamala balsam was 0.15% and the balsam has a melting point of 91 °C.

Table 1. Effect of interaction of induction techniques; on the twigs by making incision (TI), on the branches by making holes using an electric drill (BD), and on the branches by making incision (BI) and chemical stimulants; ethephon and methyl jasmonate to Rasamala balsam exudation.

Stimulant	Concentration (% (w/w))	Rasamala balsam induced (gram)		
		on the twigs with injury (TI)	on the branches by making an incision (BI)	on the branches by making holes using an electric drill (BD)
Ethephon	0.1	0.28 ± 0.05^{k}	1.71 ± 0.60^{hij}	1.24 ± 0.60^{ijk}
Ethephon	1	0.52 ± 0.08^{k}	2.18 ± 0.74^{fghi}	1.90 ± 0.60^{ghi}
Ethephon	2	0.66 ± 0.05^{k}	2.54 ± 0.45^{efgh}	2.24 ± 0.57^{fgh}
Ethephon	5	0.70 ± 0.06^{k}	2.97 ± 0.88^{def}	2.42 ± 0.60^{efgh}
Ethephon	10	0.73 ± 0.13^{k}	4.98 ± 0.69^{b}	2.90 ± 0.38^{defg}
Methyl Jasmonate	0.1	0.84 ± 0.43^{jk}	2.45 ± 0.71^{efgh}	1.92 ± 0.47^{ghi}
Methyl Jasmonate	1	2.26 ± 1.30^{fgh}	3.72 ± 0.73^{cd}	1.90 ± 1.09^{ghi}
Methyl Jasmonate	2	2.35 ± 0.96^{fgh}	3.93 ± 1.37^{c}	2.00 ± 0.74^{fghi}
Methyl Jasmonate	5	2.51 ± 0.67^{efgh}	5.54 ± 0.85^{ab}	3.41 ± 0.51^{cde}
Methyl Jasmonate	10	2.62 ± 0.40^{efgh}	6.22 ± 0.78^{a}	5.05 ± 0.55^{b}

Values are presented as the mean ± SD. Three-factorial ANOVA was used to compare multiple means, with different letters indicating significant variations in distances ($p < 0.05$).

4 Discussion

Like any other plant exudate, Rasamala balsam is naturally produced in response to environmental stress, such as physical injury (cuts and incisions), and insect and fungal attack, which is most likely a defensive mechanism because it protects the lesion from further infestation. Plant exudate is commercially obtained by tapping through incisions. This procedure has been reported to increase the quantity of gum arabic yields from *Acacia senegal* (L.) Willd. by up to 77.42% compared to untapped trees (Wekesa et al. 2009). However, this technique damages the tree if it is performed without appropriate techniques and calculations. In addition, Rasamala balsam can only be produced slowly and in limited quantities. Consequently, it has not been yet collected, processed, or used in Indonesia.

In this study, the holes created by the electric bore in the branches caused balsam exudation to be lower than that in the incised branches, indicating that the wider the square-shaped injury exposed the cambium, the more exuded the balsam Rasamala from the xylem adjacent to the cambium. This suggests that lesion response is closely associated with the severity of injury. However, the use of a relatively small incision area is recommended to minimize tree annihilation. In this study, the incised branches and twigs treated with methyl jasmonate and ethephon greatly improved balsam Rasamala yield. In addition, methyl jasmonate was found to be a better stimulator than ethephon. The difference in efficacy between two stimulants may be due to their abilities to regulate the different gene expressions related to secondary metabolite productions (Duan et al. 2010). External applications of methyl jasmonate and ethephon to broad-leaved and coniferous trees have been known to induce traumatic gum/resin duct formation (Hudgins et al. 2004; Saniewski et al. 2004), and to boost exudate production by tapping in pine (Neis et al. 2018). Thus, this study demonstrated that methyl jasmonate and ethephon could be used to improve the productivity of balsam Rasamala, which leads to a reduction in the size of the incision.

The diameter of twigs in the TI method was smaller than that of the branches in the BI method, resulting in lower Rasamala balsam production. This different reaction may be explained in part by the fact that young twigs have more active basal metabolism than adult plants, with increased investment in protein synthesis, development, and tissue formation (Gershenzon 1994), which could impede large carbon allocation to balsam.

The application of methyl jasmonate and ethephon to the twigs without incision (TW) did not induce balsam exudation. Our previous study showed that application of ethephon to the greenish surface of *L. styraciflua* twigs without incision induced balsam exudation, but the application of methyl jasmonate did not (Carolina and Kusumoto 2020). While the previous study used lanolin paste for the chemical treatment, this study used a liquid surfactant, Tween-80. Therefore, the amount of chemicals that permeated into the twigs may have been lower in this study. However, both studies have indicated that methyl jasmonate treatment without incision did not induce balsam production in Liquidambar trees. Further studies are needed to determine the induction mechanisms of methyl jasmonate with and without incision.

The colorless to white-pale yellow aromatic balsam produced by Rasamala tree is water-insoluble. This can be attributed to the main chemical components of balsam, triterpenoid, and oleanic acid contained in Liquidambar balsam (Courel et al. 2019; Aşkun et al. 2021). Similar to balsam derived from *L. orientalis,* it is semi-liquid, brown, adhesive, opaque, and aromatic (Ketenoglu and Kurt 2008). Liquidambar exudate has significant commercial value due to its medicinal characteristics and typically smells like vanilla (Aşkun et al. 2021). Therefore, understanding the physical and chemical features of balsam exudates is critical to optimize their use. The previous study has shown that methyl jasmonate treatment changed the terpenoid composition in needles and emitted volatiles of Norway spruce (*Picea abies* (L.) H. Karst.) (Martin et al. 2003). To accomplish effective balsam production and quality control, optimal plant hormone treatments and tapping techniques would need to be investigated. This could lead to greater utilization and broader industrial applications, as in the case of balsam in cosmetics and medicines.

5 Conclusion

To the best of our knowledge, this is the first study to report exudation induction in Rasamala using methyl jasmonate and ethephon. In conclusion, balsam exudation increased in a concentration-dependent manner as a result of the combined mechanical and chemical effects. The study findings showed that methyl jasmonate and ethephon have the potential to stimulate Rasamala balsam exudation, which requires wounding. Further investigation is needed to determine the functions of methyl jasmonate and ethephon in Rasamala balsam formation.

Declaration of competing interest

All the authors declare that they have no conflicts of interest to disclose.

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Authors' contribution

Anne Carolina led this study and received the grant. She was responsible for managing all tree induction work in the field. All the results of this study were scientifically analyzed and interpreted to produce a scientific manuscript.
Rita Kartika Sari: She was responsible for conducting laboratory studies on the physical characteristics of the balsam exudate.
Deded Sarip Nawawi: He was responsible for providing input and feedback on the drafted manuscript submitted for publication.
Effendi Tri Bahtiar: He was responsible for advising and assisting in analyzing the data obtained for scientific publication.
Dai Kusumoto: He was responsible for making intellectual contributions to revisions.

Declaration of openness of research materials, data, and code

Data is available on request from the corresponding author (a_caroline@apps.ipb.ac.id).

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