Effect of spraying of essential oils and passive modified atmosphere packaging on selected postharvest quality parameters and stem-end rot development in *Mangifera indica* (cultivar *Karthakolomban*)

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**Abstract:** *Mangifera indica* L. (cultivar *Karthakolomban*) is more prone to stem-end rot (SER) disease that causes substantial postharvest losses in quality as well as quantity. The objective of this study was to use aqueous emulsions of basil (1.6 µL/mL), clove (2.0 µL/mL), cinnamon leaf (2.0 µL/mL) and cinnamon bark (1.6 µL/mL) oils as sprays with passive modified atmosphere packaging (MAP) in low density polyethylene (LDPE) bags as a bio-safe strategy to control SER of *Karthakolomban* mango stored at 12 – 14 ºC and 85 – 90% of relative humidity. Distilled water and 0.1% (w/v) carbendazim 50WP treatments were used as negative and positive controls respectively. In-package respiratory gases were measured during storage for 8 days. Pathological, physicochemical, sensory properties and peel color of mango were assessed after ripening fruits at the end of the storage period. All essential oil treatments combined with passive MAP significantly reduced SER severity of mango more than 93% when compared to the negative control without adversely affecting peel colour and some physicochemical properties like total soluble solids and firmness. Mango fruits treated with basil, clove and cinnamon leaf oils obtained a higher preference by the sensory panel than other treatments. In-package O₂ concentration was at 3.8 – 5.8%, while CO₂ was at 4.3 – 5.0% at the end of the 8-day storage period. Respiratory gas levels attained a steady state equilibrium which resulted in extending the shelf life. Further, T1, T2 and T3 treatments can be commercialized as eco-friendly treatment strategies to be used during transportation and storage of mango in local trade within one week and for exportation via air cargo that takes approximately 2 – 3 days.

**Keywords:** Stem-end rot, In-Package MAP, Mango, Essential oil, Storage quality, Shelf life

**Introduction**

*Karthakolomban* is one of the superior cultivars of mango (*Mangifera indica* L.) popular among growers as well as consumers in Sri Lanka due to its delightful taste, unique flavour and higher contents of certain nutrients like vitamins A, B, C and fiber (Kothalawala and Jayasinghe, 2017). Postharvest deterioration caused by different pathogenic microorganisms result in mangoes with an undesirable quality and a shortened storage life, which will result in rejection of fruit from local and international markets (Alemu, 2014). Stem-end rot (SER) is a major disease caused by a group of pathogenic fungi which causes significant postharvest losses of mango in Sri Lanka and worldwide (Karunanayake et al., 2016). Pathogens endophytically colonize the inflorescence and remain dormant on the mature mango fruit until it begins to ripe (Alemu, 2014).

Application of synthetic fungicides is practiced by many growers as a primary and effective means to control SER of mango. However, use of fungicides has given rise to many issues, because of their high and acute toxicity to humans and other...
environmental problems (Tripathi and Shukla, 2009). Therefore, researchers are focusing on finding alternatives to fungicides in controlling postharvest diseases of fruits, including mango (Karunanayake et al., 2016).

Use of essential oils of many higher plants to control postharvest decay and enhance shelf life of fruits, is accepted worldwide as a sustainable and eco-friendly method. According to the United States Food and Drug Administration (FDA), essential oils are considered as Generally Recognized as Safe (GRAS) compounds (Nazzaro et al., 2017). The preliminary in vitro liquid bioassays conducted at the Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka, revealed the antifungal activity of Ocimum basilicum (basil), Syzygium aromaticum (clove), Cinnamomum zeylanicum (cinnamon) leaf and bark oils against SER associated fungi of mango at 0.8, 1.0, 1.0 and 0.8 µL/mL concentrations, respectively (Kodituwakku et al., 2020a).

Passive modified atmosphere packaging (MAP) of fresh produce involves sealing commodity in polymeric film packages to alter the respiratory gas levels in the headspace of the package. It modifies the atmosphere with low oxygen and high carbon dioxide levels which decline the respiration and ethylene production of the commodity to enhance its storage life (Abeywickrama, 2009). Treatments of essential oils in combination with passive MAP have found to be effective in controlling postharvest pathogens of fresh produce (Siriwardana et al., 2018). The objective of the present research was to study the efficacy of spray treatment with basil, clove, cinnamon leaf and cinnamon bark oils followed by passive MAP in controlling SER and enhancing shelf life of ‘Karthakolomban’ mango stored at 12 – 14 °C.

Materials and Methods

Preparation of mango fruits
Ninety-days old mature, green and healthy ‘Karthakolomban’ mango fruits with no record of pre-harvest fungicide treatment were obtained from orchards and home gardens in Gampaha District, Sri Lanka. Fruits were washed with running tap water and then in 1% (w/v) alum (potassium aluminum sulphate) (Devi Trading Company, Colombo, Sri Lanka) solution to remove latex. All fruits were allowed to drip dry for 30 minutes on a laboratory bench (Siriwardana et al., 2017).

Preparation of treatments
Basil oil was purchased from Aromatica Laboratories (Pvt.) Ltd., Colombo, Sri Lanka. Clove oil and cinnamon leaf and bark oils were purchased from Citro Essential Oils (Pvt.) Ltd., Mt. Lavinia, Sri Lanka. Emulsions of each test essential oil were prepared based on the minimum lethal concentration (MLC) of a particular oil determined from in vitro liquid bioassays conducted by Kodituwakku et al., (2020a). Each oil was added to distilled water (100 mL) to prepare oil emulsions with a drop of Tween 80. Carbendazim 50WP fungicide (Hayleys Agriculture Holdings Ltd., Colombo, Sri Lanka) (0.1% w/v) solution was used as the positive control (Abeywickrama et al., 2009).

Application of treatments
Prepared mango fruits were sprayed separately with the oil emulsions, positive control (carbendazim 50WP) and negative control (distilled water). The excess solutions were allowed to drain. Each fruit was placed separately in a low density polyethylene (LDPE) bag (84 gauge/21 microns) of 30.5 × 22.4 cm² surface area (Burhani Poly Films Pvt. Ltd., Colombo, Sri Lanka) and mouth of the bag was sealed using a polythene sealer (Impulse Sealer with Magnet, Mercier Corporation, Model: ME-300HCG). All treated and control samples were placed separately in ventilated corrugated 3-ply fiberboard boxes (65 × 35 × 18 cm³) lined with perforated Manila paper (60 µm). Each box comprised of six fruits. All treatments and controls were stored at 12 – 14 °C in a Walk-in cold room (Iceman Technologies (Pvt.) Ltd., Sri Lanka) with a relative humidity (RH) of 85 – 90% for 8 days (Abeywickrama et al., 2009). The experiment was repeated once under identical conditions.

In-package gas analysis
In-package respiratory gas (O₂ and CO₂) contents within LDPE bags were measured on the initial day and then on the 4th and 8th day of cold storage using
an Oxygen and Carbon Dioxide Head Space Analyzer (Model: 902 D, Quantek Instruments, Grafton, MA) (Siriwardana et al., 2017). Five replicate measurements were taken per treatment.

### Induced ripening of mango
After cold storage of 8 days, mango fruits were subjected to induced ripening at room temperature (28±2 °C) by exposing to ethylene derived from Ethepone (2-chloroethyl phosphonic acid) (Ester, Summer Field Chemicals Pvt. Ltd., Horana, Sri Lanka) for 2 days until mango attained the fully ripe stage (Abeywickrama et al., 2009).

### Assessment of pathological parameters
The SER disease severity of five randomly selected ripened mango fruits from each treatment was visually recorded as percentage SER by comparing with a SER disease severity index for 'Karthakolomban' mango developed at the Department of Plant and Molecular Biology, University of Kelaniya (Kodituwakku et al., 2020b).

### Assessment of physicochemical properties
Five randomly selected ripened mango fruits from each treatment were analyzed for physicochemical properties. Total soluble solids (TSS) (°Brix) of the filtrates of fruit pulp were determined using a handheld refractometer (ATC-1E, ATAGO Co. Ltd., Japan). Titratable acidity (TA; % citric acid) was assessed by a titration of the filtrates with 0.1 M NaOH using phenolphthalein as the indicator. pH of the filtrates was measured using a portable pH meter (PC 510, EUTECH Instruments, Singapore). Firmness of the fruit pulp was measured using a fruit firmness tester (FT 011, QA Suppliers, Italy) (Siriwardana et al., 2017).

### Assessment of peel colour
Peel colour was visually assessed using a peel colour index for 'Karthakolomban' mango developed at the Department of Plant and Molecular Biology, University of Kelaniya (1 = Fully green, 2 = Breaker, 3 = More green than yellow, 4 = More yellow than green, 5 = Fully yellow) (Kodituwakku et al., 2020b).

### Assessment of sensory parameters
Five randomly selected ripened mango fruits from each treatment were provided to a ten-member untrained sensory panel to evaluate flesh colour, aroma, texture, taste, flavour and overall acceptability. Each sensory parameter was scored as follows: excellent = 9 – 10, good = 6 – 8, fair = 4 – 5, poor = 1 – 3 (Siriwardana et al., 2017).

### Statistical analysis
Data obtained for in-package gas analysis was analyzed using Two-way ANOVA. Results with regard to physicochemical properties were analyzed using One-way ANOVA. Mean separation was done using Tukey’s multiple comparison test. Kruskal Wallis non-parametric test was used to analyze data with respect to pathological properties, sensory properties and peel colour (Siriwardana et al., 2017).

### Results and Discussion

#### In-package gas analysis
During the in-package gas analysis of passive MA packed mango, O₂ content in all treated and control packages were within the range of 20.4 – 20.6% and CO₂ content was within 0.1 – 0.2% on the initial day. A decreasing trend of % O₂ along with an increasing trend of % CO₂ was evident during the storage in the cold room at 12 – 14 °C. A distinct decline in O₂ was observed in all packages on the 4th day of storage (i.e. 3.8 – 5.9%) accompanied by a dear decrease in CO₂ (i.e. 3.6 – 4.4%) when compared to the initial gas levels. At the end of 8-day storage period, final values were between 3.8 – 5.8% of O₂ and 4.3 – 5.0% of CO₂ (Figure 1). These results revealed that a steady state equilibrium has been achieved by the in-package respiratory gases during the 8 days storage time. According to the two-way ANOVA, O₂ content in the packages of different treatments were significantly different from the controls when treatment method was considered as a factor (P<0.05) indicating treatments having an effect on O₂. Similarly, O₂ contents of different treatments were significantly different from the controls when storage time was considered as a factor (P<0.05). Oxygen in packages declined with storage (Figure 1A). Moreover, O₂ contents of different treatments were significantly different when the interaction of treatment × storage time was considered as a factor (P<0.05). The CO₂ levels in the packages of treatments and controls were significantly different only when treatment method and storage time were considered as separate factors (P<0.05). However, there was no statistically significant difference in % CO₂ when the interaction of treatment × storage time was considered as a factor (P>0.05). This may be due to levelling off of CO₂ with time of storage after 8 days as seen in Figure 1B.

When O₂ consumption by the produce equals O₂ diffusion into the package and CO₂ production by the
produce equals CO\textsubscript{2} diffusion out of the package, two gases attain steady state equilibrium. This equilibrium levels of O\textsubscript{2} and CO\textsubscript{2} could alter enzymatic activities related with respiration which influence the expansion of shelf life of the MA packed commodity. Also, modified atmospheric conditions could hinder the ethylene biosynthesis which delays ripening of the commodity (Siriwardana \textit{et al.}, 2018). As suggested by Siriwardana \textit{et al.} (2018), the extended 8 days shelf life of ‘Karthakolomban’ mango could be due to the in-package modified respiratory gas levels under equilibrium. According to Illeperuma and Jayasuriya (2002), O\textsubscript{2} and CO\textsubscript{2} levels in LDPE packages containing ‘Karthakolomban’ mango stored at 13 °C varied between 13.4 – 3.7% and 3.0 – 10.2%, respectively, from the 14\textsuperscript{th} day to 27\textsuperscript{th} day of storage. Further, present findings are in agreement with Illeperuma and Jayasuriya (2002), since both studies highlight decreasing and increasing trends of O\textsubscript{2} and CO\textsubscript{2} respectively in the headspace of passive MA packed mango. Present results are also in conformity with Siriwardana \textit{et al.} (2018) which showed similar trends for respiratory gas changes in basil oil treated, passive MA packed ‘Embul’ banana.

![Graphs showing oxygen (A) and carbon dioxide (B) levels of passive modified atmosphere packed ‘Karthakolomban’ mango sprayed with different essential oils and stored at 12 – 14 °C for 8 days. Each data point represents the mean (±standard error) of 10 replicates.](image)

**Figure 1.** Oxygen (A) and carbon dioxide (B) levels of passive modified atmosphere packed ‘Karthakolomban’ mango sprayed with 1.6 µL/mL basil oil (T1), 2.0 µL/mL clove oil (T2), 2.0 µL/mL cinnamon leaf oil (T3), 1.6 µL/mL cinnamon bark oil (T4), distilled water (T5) and 0.1% (w/v) carbendazim (T6) and stored at 12 – 14 °C for 8 days. Each data point represents the mean (±standard error) of 10 replicates.

**Pathological assessments**

The highest SER severity (\textit{i.e.} 15.1%) was observed in the negative control samples treated with distilled water after induced ripening. Mango subjected to spray treatment with different essential oils displayed % SER values within the range of 0.3 – 1.1%, which are notably lower when compared to the negative control. Carbendazim 50WP treated mango exhibited no SER (Figures 2 and 3). Disease severity of all essential oil treated mango and the positive
Control of postharvest quality and stem-end rot in mango

Control were found to be significantly lower than the negative control (p<0.05). Furthermore, essential oil and fungicide treatments did not show a significant difference in reducing SER severity and this indicates the equal efficacy of essential oil and fungicide treatments in controlling SER.

Figure 2. Stem-end rot disease severity of passive modified atmosphere packed ‘Karthakolomban’ mango sprayed with 1.6 µL/mL basil oil (T1), 2.0 µL/mL clove oil (T2), 2.0 µL/mL cinnamon leaf oil (T3), 1.6 µL/mL cinnamon bark oil (T4), distilled water (T5) and 0.1% (w/v) carbendazim 50WP (T6) after storage at 12 – 14 °C for 8 days and subjected to induced ripening. Each data point represents the mean of 10 replicates. Means sharing a common letter(s) are not significantly different by Kruskal Wallis non-parametric test (P<0.05).

Figure 3. Appearance of ‘Karthakolomban’ mango subjected to spray treatment + MAP, stored at 12 – 14 °C for 8 days and after induced ripening. (A) Control, (B) 1.6 µL/mL basil oil, (C) 2.0 µL/mL clove oil, (D) 2.0 µL/mL cinnamon leaf oil, (E) 1.6 µL/mL cinnamon bark oil and (F) 0.1% (w/v) carbendazim 50WP.

According to Karunanayake et al. (2018), cardamom oil at 0.70 µL/mL has significantly reduced SER of ‘Karthakolomban’ mango during a dip treatment. This result is in accordance with the present findings because different essential oils having similar antifungal components could control SER of
'Karthakolomban' mango at low concentrations. The gas chromatography-mass spectroscopy analysis of the essential oils selected for the present study identified eugenol, methyl chavicol, cinnamaldehyde and linalool as the major antifungal chemical components (Kodituwakk et al., 2020a). These components are capable of inhibiting the growth and multiplication of microbial cells by implementing different mechanisms of action at cellular level (Zaker, 2016). Further, several antifungal components present in a single oil, in different proportions may act synergistically to inhibit the growth of target fungi (Anthony et al., 2004). The atmosphere saturated with water in MA packages might be another factor that could delay the deterioration of mango. High humidity inside the packages reduces the transpiration, hence controlling shrivelling of fruits. Further, the film used for packaging reduces the dissipation of treated essential oils and creates a micro-atmosphere of essential oil vapour inside the package to control the target pathogens over a prolonged period (Ben-Yehoshua, 1985).

Physicochemical properties
Certain treatments displayed a significant difference in physicochemical parameters with respect to the untreated negative control (p>0.05). The TSS of treatment and control samples were within the range of 27.3 – 35.7 °Brix, while TA was at 0.5 – 1.1%. pH and firmness of all samples were at 2.8 – 4.2 and 0.6 – 0.8 kg cm$^{-2}$ respectively (Table 1). However, some treatments did not adversely alter the physicochemical properties of mangoes by affecting their postharvest quality.

Table 1. Physicochemical properties of 'Karthakolomban' mango subjected to spray treatment plus passive MAP, stored at 12 – 14 °C for 8 days and subjected to induced-ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSS1 (°Brix)</th>
<th>TA2 (% citric acid)</th>
<th>pH</th>
<th>Firmness (kg cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>27.3$^b$</td>
<td>1.0$^a$</td>
<td>3.2$^{cd}$</td>
<td>0.7$^{ab}$</td>
</tr>
<tr>
<td>T2</td>
<td>34.8$^c$</td>
<td>0.5$^b$</td>
<td>4.2$^a$</td>
<td>0.7$^{ab}$</td>
</tr>
<tr>
<td>T3</td>
<td>35.7$^a$</td>
<td>0.6$^a$</td>
<td>4.0$^{ab}$</td>
<td>0.6$^c$</td>
</tr>
<tr>
<td>T4</td>
<td>30.2$^{ab}$</td>
<td>0.6$^a$</td>
<td>3.5$^{bc}$</td>
<td>0.6$^c$</td>
</tr>
<tr>
<td>T5</td>
<td>34.5$^a$</td>
<td>1.1$^a$</td>
<td>2.8$^d$</td>
<td>0.7$^{ab}$</td>
</tr>
<tr>
<td>T6</td>
<td>34.1$^a$</td>
<td>1.1$^a$</td>
<td>3.0$^d$</td>
<td>0.8$^a$</td>
</tr>
</tbody>
</table>

T1: 1.6 µL/mL basil oil, T2: 2.0 µL/mL clove oil, T3: 2.0 µL/mL cinnamon leaf oil, T4: 1.6 µL/mL cinnamon bark oil, T5: control and T6: 0.1% (w/v) carbendazim 50WP. °Total Soluble Solids; ºTitratable Acidity; Each data point represents the mean of ten replicates. Within a column, means followed by the same letter are not significantly different by the Tukey's pair-wise comparison test (P=0.05).

Sefu et al. (2015) and Gunasekera et al. (2018) reported that TSS values of cinnamon leaf and bark oil treated mango were significantly lower when compared with the control. However, present study is not in agreement with Sefu et al. (2015) and Gunasekera et al. (2018) since a significant difference in TSS was not observed among samples treated with cinnamon oils and controls. TA levels of cinnamon oil treated mango reported by Sefu et al. (2015) (i.e. 0.24% TA) and Gunasekera et al. (2018) (i.e. 0.31% TA) were comparatively lower than the values demonstrated by cinnamon oil treated mango in the present study. However, pH values of mango subjected to cinnamon oil treatments were somewhat similar to the pH values reported by Sefu et al. (2015) and Gunasekera et al. (2018) (i.e. around 3.8). Since the firmness of mango treated with cinnamon leaf oil was significantly different from the controls, present study is in accordance with Sefu et al. (2015) who have reported a similar observation. Nevertheless, Gunasekera et al. (2018) reported no significant difference in firmness of cinnamon bark oil treated mango when compared with the control. Therefore, present study is not in conformity with Gunasekera et al. (2018) because firmness of mango subjected to cinnamon bark oil treatment was significantly different from the control samples.

According to Illeperuma and Jayasuriya (2002), passive MAP of 'Karthakolomban' mango has not drastically altered TSS, TA, pH and firmness, when compared to the control. Therefore, results of the present study are in agreement with Illeperuma and Jayasuriya (2002) since passive MAP has not adversely affected the physicochemical properties of most treatments during the present study. Variations in the postharvest behavior of fruits are caused due to their slight maturity differences and this could result in slight changes in certain physicochemical properties (Anthony et al., 2003). Consumers usually prefer sweeter mango with less acidic taste. Jayawickreme (2012) reported that the sweetness of ripe mango is related with more TSS and higher pH. Therefore, mango treated with cinnamon leaf oil could have a better consumer
preference since it resulted in a higher TSS, lower TA and higher pH when compared to most of the other treatments. Errors could be minimized by replication and in this study, each treatment was replicated 10 times.

Peel colour
After the storage period of 8 days at 12 – 14 °C, mango was at the ‘fully green’ (index value = 1) or ‘colour break’ (index value = 2) stages. Peel colour of mango belonging to all treatments and control appeared within the range of 4.50 – 4.90 after induced ripening and all fruits were at the stages of ‘more yellow than green’ (index value = 4) or ‘fully yellow’ (index value = 5). Any significant difference in peel colour after induced ripening was not observed among all treatments and control (P>0.05). Nevertheless, present finding is not in conformity with Karunanayake et al. (2018) who reported that basil oil treatment (i.e. 0.70 µL/mL) has previously significantly altered the peel colour of ‘Karthakolomban’ mango giving an unusual appearance.

Table 2. Sensory properties of ‘Karthakolomban’ mango subjected to spray treatment plus passive MAP, stored at 12 – 14 °C for 8 days and subjected to induced-ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flesh colour</th>
<th>Aroma</th>
<th>Texture</th>
<th>Taste</th>
<th>Flavour</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>6.9a</td>
<td>6.7a</td>
<td>6.8a</td>
<td>6.6a</td>
<td>6.6a</td>
<td>6.9a</td>
</tr>
<tr>
<td>T2</td>
<td>7.2a</td>
<td>6.3a</td>
<td>6.7a</td>
<td>6.6a</td>
<td>6.7a</td>
<td>6.7a</td>
</tr>
<tr>
<td>T3</td>
<td>7.1a</td>
<td>6.1a</td>
<td>6.7a</td>
<td>6.7a</td>
<td>6.6a</td>
<td>6.8a</td>
</tr>
<tr>
<td>T4</td>
<td>7.4a</td>
<td>6.0a</td>
<td>6.1a</td>
<td>5.4b</td>
<td>5.3b</td>
<td>5.6b</td>
</tr>
<tr>
<td>T5</td>
<td>6.6a</td>
<td>6.2a</td>
<td>6.3a</td>
<td>5.2b</td>
<td>5.3b</td>
<td>5.6b</td>
</tr>
<tr>
<td>T6</td>
<td>6.3a</td>
<td>5.8a</td>
<td>5.7a</td>
<td>5.1b</td>
<td>5.1b</td>
<td>5.6b</td>
</tr>
</tbody>
</table>

(T1: 1.6 µL/mL basil oil, T2: 2.0 µL/mL clove oil, T3: 2.0 µL/mL cinnamon leaf oil, T4: 1.6 µL/mL cinnamon bark oil, T5: control and T6: 0.1% (w/v) carbenazim. Each data point represents the mean of twenty replicates. Within a column, means sharing the same letter are not significantly different by the Kruskal Wallis non-parametric test (P=0.05).

When pathological, physicochemical and sensory properties of mango subjected to essential oil treatments plus passive MAP are considered together, those treatment systems could be further improved and introduced to the growers and suppliers to provide good quality mango to the market. As the disease severity of mango treated with essential oils was less than 1%, treatments need to be further developed to accomplish the complete inhibition of SER. Further, having mature, green and firm mango without any physiological disorders or disease symptoms is important to reduce the postharvest losses that could occur during transportation and storage (Sarananda and Amarakoon, 1999). Therefore, the present treatment methods need to be improved to achieve a lengthened storage life of mango requiring more than 8 days for consideration for long distance transportation and export.

Conclusion
Spray treatment with emulsions of basil (1.6 µL/mL), clove (2.0 µL/mL), cinnamon leaf (2.0 µL/mL) and cinnamon bark (1.6 µL/mL) oils followed by passive MAP in LDPE bags efficiently controlled SER of ‘Karthakolomban’ mango up to an acceptable level during the storage at 12 – 14 °C for 8 days without unfavourably affecting some physicochemical properties and peel colour of mango. However,
sensory panel had a somewhat higher preference towards basil, clove and cinnamon leaf oil treated mango. The modified atmosphere developed in LDPE packages with the appropriate levels of O₂ and CO₂ was desirable for storage of mango at low temperature. Further, T1, T2 and T3 treatments can be commercialized as eco-friendly treatment strategies to be used during transportation and storage of mango in local trade within one week and for exportation via air cargo that takes approximately 2 – 3 days.

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References


Kodituwakku et al.