Antifungal activity of essential oil against *Botrytis cinerea* in *Vitis vinifera L*

Reshni Maisnam¹, Yingmien L Antuhu ¹, Christina VL Muanpuii, Asha Kumari ², Jorge Rojas Lopez Menchero³, Azucena Gonzalez Coloma³*, Marie Fe Andres³, Nutan Kaushik¹,²*

¹Amity Institute of Horticulture Studies and Research, Amity University Uttar Pradesh, Noida, India, 201303  
²Amity Food & Agricultural Foundation, Amity University Uttar Pradesh, Noida, Uttar Pradesh, India, 201303  
³Institute of Agricultural Sciences, CSIC, Madrid, Spain  
*Corresponding author: nkaushik5@amity.edu, azu@ica.csic.es

Abstract. Grapes (*Vitis vinifera L*) is extensively cultivated and widely consumed fruit world wide But it is also very highly susceptible to fungal infections such as *B cinerea* which increases post-harvest losses, quality deterioration, and making it unsuitable for human consumption. This results in significant economic loss of grapes and impacts grape farmers' profits. Therefore, there is an urgent need to identify potential methods and approaches to decrease post-harvest losses of grapes caused by *B. cinerea*. Currently, synthetic fungicides are used to control grape infections which has harmful impact on the environment and human health. Therefore, essential oil from plants sources could be a potential alternative to synthetic fungicides. In this study, antifungal activity of three essential oils was evaluated against *B. cinerea* using fumigation method. In an in vitro study, the essential oils extracted from *Zingiber officinale* and *Saturaga montana*, each at a concentration of 4mg/mL, exhibited complete inhibition (100%) of fungal growth. The physiological study of fruit in term of TSS, pH and weight loss showed that there is slow deterioration of fruit during storage. In-vivo results showed that essential oil from natural source can be potential alternative for synthetic fungicides which is ecofriendly and effective to control diseases and post-harvest losses in grapes.

Keywords: *Vitis vinifera, Botrytis cinerea, Fumigation method, Synthetic fungicides, Essential oil*

1 Introduction

Grapes (*Vitis vinifera L*) are one of the most important fruit crops which have many health benefits. Such as it help in improving heart health, is rich in antioxidants, improves skin, boosts immunity, and helps the body to recover quickly. Maharashtra, Karnataka, Tamil Nadu, and Andhra Pradesh are major cultivators of grape in India [1]. On the other hand, the grape industry in India faces challenges in controlling post-harvest losses of grapes due to fungal diseases caused by *Botrytis cinerea* such as, Downey mildew, Powdery mildew, and bacterial pathogen like *Xanthomonas campestris*. Fungal infection on grapes results in economic loss, low quality of grapes, profit of grapes farmers. Traditional methods of disease control in grapes crop are chemical pesticides which causes harmful impact on environment, human health and increases the emergence of pesticides resistant pathogens. Therefore, efficient, sustainable disease management strategies must be identified to control post-harvest grape losses in India [2]. Essential oil from *Azadirachta indica*, *Thymus vulgaris* and tea tree oil showed anti-fungal activity against *B. Cinerea*. The essential oil from natural sources could be promising, alternatives and sustainable approaches for disease management in grapes. Applications of essential oils from natural sources having bioactive properties such as anti-microbial, anti-fungal and insecticidal properties) have attracted the attention of researchers.

This research aims to study the efficacy of essential oils as an alternative method for *B. cinerea* control in grapes. This study focuses to identify sustainable and environmentally friendly strategies to mitigate post-harvest losses and enhance the overall quality and marketability of Indian grapes.

2 Materials and methods

2.1 Raw material

All the experiments were conducted on a healthy and mature of Timpson table grape berries procured from a supermarket in Madrid, Spain. Essential oils obtained from *Zingiber officinale*, *Piper nigrum* were purchased from the commercial market in India and *Saturaga montana* were obtained from CSIC lab and used to check antifungal activity.
2.2 Inoculum preparation

The inoculum was prepared with spores of *Botrytis cinerea* obtained from 15-20-day-old culture. 500 uL of spores were suspended in 500 uL of sterile distilled water. The final concentration was adjusted to $10^6$ spores /mL [5].

2.3 In-vitro study of antifungal activity of the essential oils against *B. cinerea*

The antagonistic activity of essential oils obtained from different plant species against bunch rot disease in Timpson grapes caused by *Botrytis cinerea* was evaluated using fumigation method [6,7]. The fumigation was carried using *Z. officinale*, *P. nigrum* and *S. montana* essential oils (each at concentration 4mg/mL). Potato Dextrose Agar (PDA) was poured in 4 wells of multi well titre plate. The spores of *Botrytis cinerea* (5uL of a spore solution with a concentration of $1x10^6$ spore per mL) was poured into the centre of the 4 wells containing PDA and kept the middle well of plate empty to put the cellulose disc with essential oils. 250µL of the essential oil from the stock solution of essential oil was taken in a cellulose disk (approximately 2cm diameter) and placed in the centre of middle well for the treatment and 250 uL of ethanol was used for control. The plates were sealed using para film and incubated at 25°C until the control achieves a maximum diameter (approximately 1.8 to 2 cm). The inhibition percentage of fungal growth was measured using the formula [8].

\[
\text{Inhibition} (\%) = 100 \times \frac{G_c - G_t}{G_c} 
\]

Where,

- $G_t$: Diameter of fungal growth using essential oil (cm)
- $G_c$: Diameter of fungal growth using Ethanol (cm)

2.4 In-Vivo assay of selected essential oil (*Satureja montana*) against *Botrytis cinerea*

The surface sterilization of grapes was done using 1% sodium hypochlorite solutions, followed by immersion in 100% ethanol, and then rinse with sterilized distilled water[9]. An essential oil (*Saturaga montana*) was selected for In-vivo study because *Saturaga montana* showed highest inhibition percentage against *B. cinerea* in vitro study. 250 uL of *Saturaga montana* (25mg/mL concentration) was taken in a cellulose disk. The disk (6.25 mg/mL) was pasted on lid of 125 mL jar. The grapes were punctured (approximately 0.2cm) and 5uL of spore solutions containing $2x10^6$ spores / mL applied on the wound. Ethanol is used as a control. The lid of jar was closed and incubated at 25°C for 7-10 days. Observations were taken at every 24-hour intervals.

2.5 Determination of quality of grapes during storage

The assessment of Timpson grapes’ quality after post-treatment with the essential oil was evaluated on the first and last day (after 7 days of storage) based on weight loss percentage, fruit firmness, total soluble solids, and pH.

\[
\text{Weight loss} (\%) = \frac{W_0 - W_T}{W_0} \times 100
\]

Where,

- $W_0$: Initial weight (g)
- $W_T$: Final weight (g)

Fruit firmness assessments were performed by penetrometer [10]. Total soluble solids were measured in grapes juices at ambient temperature using digital refractometer. The pH of grape juices was measured at room temperature using pH meter [11].

2.6 Statistical analysis

Microsoft Excel 2010 and IBM SPSS version 29.0.2 were Used for statistical analysis and data processing in this study. The data are presented in mean ± standard deviation of two replications

3 Results and discussion

Essential oils from the natural source were selected for this study due to their broad-spectrum antimicrobial activity. According to previous studies, these essential oils were reported to produce volatile compounds showing antimicrobial activity against *B.Cinerea*. Therefore, essential oils extracted from these plant species may be capable of producing volatile compounds that suppress the bunch rot disease caused by *B. Cinerea* in post-harvest grapes.

3.1 In vitro anti-fungal activity of essential oil against mycelial growth of *B. cinerea*

In the in vitro study, the anti-fungal activity of essential oil of *Zingiber officinale*, *Piper nigrum* and *Saturaga montana* against the growth of *B. cinerea* was studied using fumigation method. In this study all the essential oil showed 100 % inhibition of fungal growth as compared to control (Table 1 & fig. 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturaga montana</td>
<td>100 ±0.0NS</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>100 ±0.0NS</td>
</tr>
<tr>
<td>Piper nigrum</td>
<td>100±0.0NS</td>
</tr>
</tbody>
</table>

Note: NS indicates no significant difference between the treatments (p>0.05)
In vitro assay of essential oil against *Botrytis cinerea* at concentration of 4mg/ml (A) Control (ethanol) (B) *Zingiber officinale* essential oil (C) *Saturaga montana* essential oil (D) *Piper nigrum*.

### 3.2 In vivo study of anti-fungal activity of essential oil on grapes

The essential oil showing the highest antifungal activity was selected for the in vivo analysis in grapes as shown in fig. 2. The grapes berries were inoculated with *B. cinerea* spores *and* treated with *Saturaga montana* less deteriorated than the control after 7 days of incubation. The effects of essential oil on the quality of grape berries during 7-day storage were assessed in terms of weight loss percentage, firmness, total soluble solids, pH. Significant water loss and physical change was observed in grape berries during storage in all conditions. After seven days of storage, no significant difference was observed in pH of the grape berries (Table 2). But significant difference was observed in weight loss (Fig. 3), firmness, colour and change in the appearance of grapes was observed which showed that grapes treated with essential oil were spoiling with slowly as compared to control. Therefore essential oil could be used to control diseases caused after post harvest of grapes.

#### Table 2. Physical & Chemical analysis of grapes during 7 days storage

<table>
<thead>
<tr>
<th>Storage Day</th>
<th>Firmness (N)</th>
<th>TSS (°Brix)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAY 1</td>
<td>1.3</td>
<td>10.0</td>
<td>3.60</td>
</tr>
<tr>
<td>DAY 7</td>
<td>2.0</td>
<td>15.0</td>
<td>3.65</td>
</tr>
</tbody>
</table>

#### Fig. 3. Weight loss (%) of grapes berries after 7 days of storage. B+EO: grapes treated with *Saturaga montana*, Control: ethanol. Data are presented as mean ± Standard deviation. Different lowercase letter indicate significant difference between the treatments (p<0.05) in which a is highest.

### 4 Conclusion

Grapes (*Vitis vinifera L*) is widely consumed fruits all over the world due to many health benefits such immunity boosting, control cardiovascular diseases and helps body to recover quickly. But grapes are highly perishable fruit which result in lower quality fruit and grapes farmers suffer huge economic loss due to post harvest loss of the grapes. This study indicates that essential oils from different plant sources was showing inhibitory effects against *Botrytis cinerea*, a common fungal pathogen affecting grapes during storage. Among three essential oils, *Zingiber Officinale* and *Saturaga Montana* essential oil showed highest antifungal activity against *B cinerea*. Based on in vitro assay, *Saturaga Montana* essential oil was selected for in-vivo study. The result suggest that essential oils from *Zingiber Officinale* and *Saturaga Montana* shows the highest inhibition and can be used as a natural alternative for synthetic fungicides.

### Conflict of interest

The authors declare no conflict of interest.

### References

2. FAOSTAT. FAO database for food and agriculture; [Cited 2020 May 15], 2019


