

Novel *Prosopis juliflora* leaf ethanolic extract as natural antifungal agent against *Botrytis cinerea*: application on strawberries shelf-life extension

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1. ABSTRACT

Introduction: Fresh produce's spoilage is a worldwide concern that accompany the global increase in food demand. Adverse human health and environmental effects of commercial pesticides is a major public concern.

Material and Methods: In this report, our *Prosopis juliflora* water soluble leaves ethanolic (PJ-WS-LE) extract will be described as an effective antifungal agent.

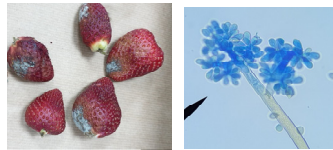
Results: PJ-WS-LE extract showed total inhibition of *Botrytis cinerea* growth and extend strawberries shelf-life.

Future directions: PJ-WS-LE extract will be chemically described and its effectiveness on other fresh produce will be evaluated.

2. BACKGROUND

Botrytis cinerea is one of the top ten pathogens that affect fresh produce shelf-life around the world. With a global effort to control *B. cinerea* grey mold, countries spend a lot of money on botryticides, wide spectrum fungicides and bio-controllers. *Botrytis cinerea* can progress easily from infected fruits to healthy ones even at low storage temperatures which can lead to entire lots spoilage in few weeks.

Prosopis juliflora has been explored for its antimicrobial activities. However, studies are not interconnected.



Strawberries are widely consumed raw berries which are famous in their processed forms such as jam and juices. The delicate fruit has a very short postharvest life, it is susceptible to mechanical injuries, fast dehydration and fungal infection. Strawberries' most common spoiling fungi is *B. cinerea*. Our PJ-WS-LE extract is a promising fungicides alternative that showed *in-vitro* effectiveness against *B. cinerea*.

3. OBJECTIVES

- Identifying a natural plant extract bio-controller.
- Controlling *B. cinerea* fungal postharvest spoilage.
- Extending strawberries shelf-life.
- Maintaining strawberries quality parameters during storage.

4. METHODOLOGY

4.1 Disk antifungal method

Used to test the effect of 20mg/ml of PJ-WS-LE extract in PDA plate on *B. cinerea* growth compared to normal growth.

4.2 Determination of minimum inhibitory concentration

96-well plate method was used to determine PJ-WS-LE extract MIC against *B. cinerea*.

4.3 Mode of action

The inhibited *B. cinerea* disk was transferred to a clean PDA plate to determine the extract mode of action.

4.4 Effect of PJ-WS-LE extract on the microscopic morphology of *B. cinerea* using SEM

Cultures of *B. cinerea* were prepared in the presence & absence of 8mg/ml of PJ-WS-LE extract. SEM pictures of spores and hyphae were taken.

4.5 Determination of shelf-life of strawberry samples

Treated strawberry samples (8mg/ml PJ-WS-LE extract) and control samples had their shelf-life determined at room temperature and at 4°C.

4.6 Strawberries storage quality parameters monitoring

Quality parameters of treated and control samples were monitored on a weekly basis: weight loss, bacterial and fungal CFU, firmness, pH and TSS.

6. RESULTS

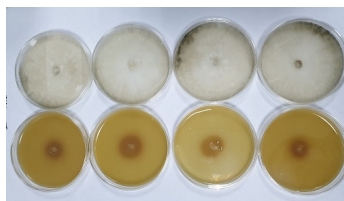


Fig 1. Disk antifungal methods results of *B. cinerea*

PJ-WS-LE extract has fungicidal effect on *B. cinerea*.

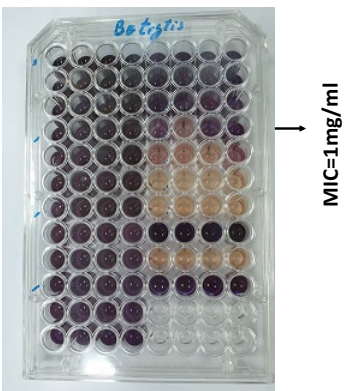
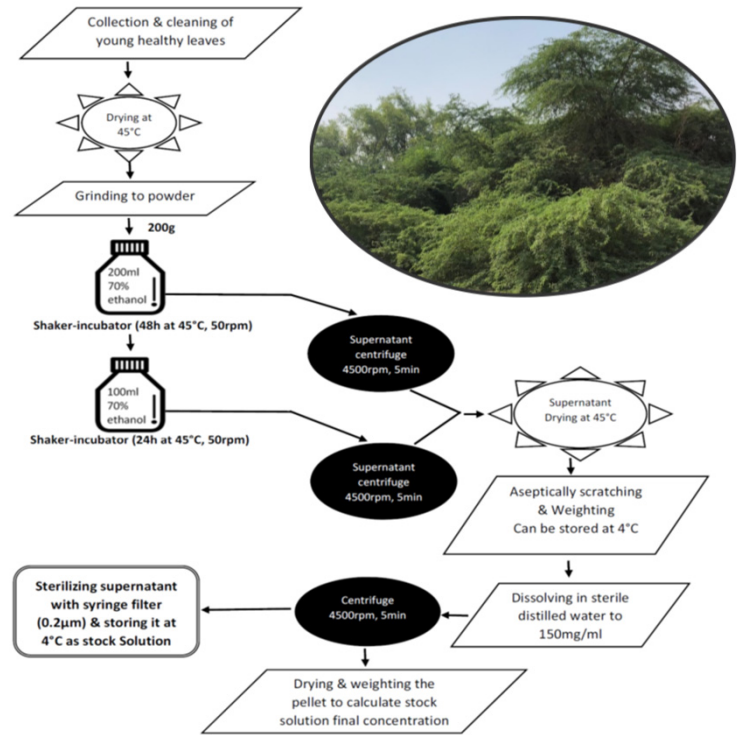


Fig 2. 96-well plate results of *B. cinerea*

5. NOVEL EXTRACT PREPARATION METHOD



6. RESULTS & DISCUSSION

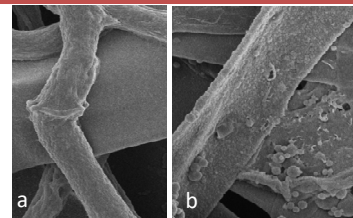


Fig 3. SEM pictures of *B. cinerea* hyphae (control (a)-Treated (b))

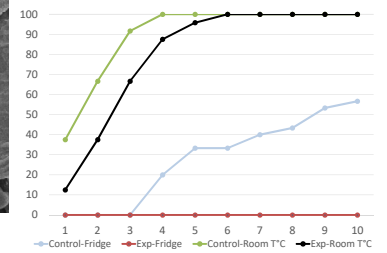


Fig 4. Percentage of strawberry samples that rotten every day.

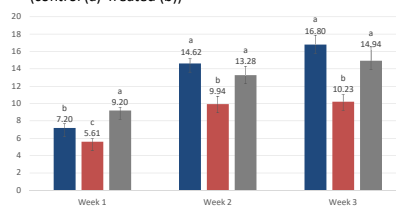


Fig 5. Weekly weight loss percent (Control (A), coated with 8mg/ml PJ-WS-LE extract (B), coated with 1% chitosan (C)).

Table 1. Weekly quality parameters

Treatment Batch	Storage	Storage		
		Firmness (N)	pH	TSS (Brix)
Batch A negative control samples	1	29.86 ± 3.5	3.61 ± 0.071	61.2 ± 3.1
	2	26.24 ± 3.1	3.77 ± 0.052	69.0 ± 3.5
	3	20.83 ± 2.1	3.73 ± 0.061	67.7 ± 3.2
Batch B coated with 8mg/ml PJ-WS-LE extract	1	29.53 ± 1.1	3.69 ± 0.033	61.2 ± 2.6
	2	32.42 ± 3.2	3.66 ± 0.028	65.8 ± 4.4
	3	30.59 ± 3.8	3.74 ± 0.037	63.0 ± 1.3
Batch C coated with 1% chitosan	1	34.22 ± 4.4	3.65 ± 0.057	65.2 ± 5.9
	2	35.10 ± 6	3.65 ± 0.042	63.2 ± 4.5
	3	33.35 ± 2.9	3.67 ± 0.055	63.0 ± 4.6

7. CONCLUSIONS

- PJ-WS-LE totally inhibits the growth of *B. cinerea* with fungicidal activity.
- MIC of PJ-WS-LE extract against *B. cinerea* is 1mg/ml.
- Coated strawberries (8mg/ml PJ-WS-LE extract) had their shelf-life at 4°C extended from 4.3 days to 10 days.
- 8mg/ml PJ-WS-LE extract coating is more effective than 1% chitosan.
- PJ-WS-LE extract showed promising fungicidal characteristics that allow it to replace chemical fungicides.

8. REFERENCES

Iman Saleh and Mohammed Abu-Dieyeh. Novel *Prosopis juliflora* leaf ethanolic extract as natural antimicrobial agent against food spoiling microorganisms. Scientific Report.
Polat, I., Bayraktar, Ö., Mercati, F., Gümrükcü, E., Sütlü, G., Kitapçı, A., ... Carim, F. (2018). Characterization of *Botrytis cinerea* isolates collected on pepper in Southern Turkey by using molecular markers, fungicide resistance genes and virulence assay. *Infect Genet Evol*, 60, 151-159. doi:10.1016/j.meegid.2018.02.019.
Tomas-Grau, R. H., Hael-Conrad, V., Requena-Serra, F. J., Perato, S. M., Caro, M. d. P., Salazar, S. M., & Diaz-Ricci, J. C. (2020). Biological control of strawberry grey mold disease caused by *Botrytis cinerea* mediated by *Colletotrichum acutatum* extracts.