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Enosencia Molwantoa * and Martin Johannes Potgieter

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The Control of Powdery Mildew in Watermelon Using Homemade Fungicide Containing *Campuloclinium macrocephalum* Extract

E. Molwantoa * and M. J. Potgieter

Department of Biodiversity, University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa
* Correspondence: Enosencia7@gmail.com

Abstract: Background: Fungal plant diseases are a big problem to farmers and food markets. These infections reduce the plant's production and yield, negatively affecting the food supply. The Curcubitaceae family is very susceptible to most fungal diseases, especially powdery mildew. *Campuloclinium macrocephalum* (pom-pom weed), an invasive exotic in South Africa, has antifungal properties. **Aim**: To investigate the effectiveness of pom-pom weed extract in controlling watermelon powdery mildew (*Podosphaera xanthii*) fungal disease. **Methods**: Watermelon plants grown in plastic pots, were irrigated with a Hoagland nutrient solution. The plants were organised in a randomised block design. The treatments were: negative control, no treatment (Treatment A), positive control, plants sprayed with commercial fungicide (Treatment B), plants sprayed with buffered plant extract (Treatment C), plants sprayed with plant extract of 0.1 mg/ml (Treatment D) and plants sprayed with plant extract of 0.2 mg/ml (Treatment E). Foliar application of the different treatments on the watermelon plants was done once per week for five weeks. **Results**: The negative control (Treatment A) had the highest mildew leaf coverage at 77.69%. This was followed respectively by Treatment C at 13.10%, Treatment E at 7.56%, Treatment D at 2.05%, and lastly by Treatment B at 0%. There was a significant difference ($p<0.05$) amongst the treatments. **Conclusion**: The results of the study showed that pom-pom weed extract at a minimum concentration of (0.1 mg/ml) can be used to control powdery mildew in watermelons, thus reducing the usage of chemical fungicides.

Keywords: antifungal activity; home-made fungicide; *Podosphaera xanthii*

1. Introduction

Plant diseases, especially fungal diseases, contribute significantly to plant productivity and yield loss. About 30% of food loss is caused by plant diseases (Ajala et al., 2020), with more than 70% of the loss caused by fungi (Agrios, 2005). Consequently, an increase in the world's population has led to a surge in the use of chemicals to protect crops and meet the demand for food. Unfortunately, environmental issues limit using chemicals to control plant diseases (Wolf and Verreet, 2008), resulting in employing organic compounds from plants to control plant fungal diseases (Dosumu et al., 2019).

Powdery mildew has historically been an important fungal disease of watermelons globally, including South Africa (Brisbois et al., 2018). During growth and developmental stage of watermelons, powdery mildew is commonly a yield limiting factor (Cui et al., 2021). Powdery mildew infections are not confined to cucurbit species, but have jumped to other crop species like mushmelons, honeydew, pumpkins, squash, and gourds (Roberts and Kucharek, 2005). This disease leads to reduced yield of affected crops by limiting growth of plants and causing early loss of leaves. Thus, crop yield reduction is due to the rate of fungal infection and the spread rate on infected plants (Mossler and Neshelm, 2005). *Podosphaera xanthii* is the most common and important fungus on watermelon as it spreads faster and is more persistent than other fungi (Jahn et al., 2002). There is also an issue of resistance by pathogens to fungicides. McGrath (2001) showed the resistance of

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powdery mildew to the commonly used fungicides, benomyl and triadimefon. Moreover, there is much interest in finding alternatives that are environmentally friendly and safe to biodiversity (Chowdhury et al., 2024).

Since it was discovered that some plants produce chemicals or enzymes that can fight diseases (Balouiri et al., 2016), they have become good targets of investigation to control plant infections, including powdery mildew. The antifungal activity of plants reported can be attributed to the presence of secondary metabolites like tannins, phenolics, flavonoids, saponins, alkanoids and terpenoids (Reynolds et al., 1996) and chitinase enzyme (destroys the cell wall of the fungi) (Silva, 2016). Wurns (1999) showed that terpenoids disrupt the membrane of microorganisms, tannins lead to enzyme inhibition, while flavonoids inactivate enzymes and destroy the cell wall.

Campuloclinium macrocephalum, pompom weed, is one of the potential sources of secondary metabolites and useful bioactive compounds (McGaw et al., 2022). Pompom weed was introduced into South Africa during the early 1960s as an ornamental plant and has become a significant weed that threaten the grasslands and wetlands of South Africa (Zachariades et al., 2021). *Citrillus lanatus*, watermelon, is said to have originated from the Kalahari Desert in southern Africa (Malambane et al., 2023). With time, it became popular in most African countries. Moreover, the cultivation of watermelon spread to other countries like America and Asia (Kousik et al., 2019). The current study was designed to investigate using pom-pom weed extract to control powdery mildew on watermelons. This is done to address food security and sustainability in developing countries, by developing cheaper and more environmentally friendly alternatives to synthetic applications.

2. Materials and Methods

Bitter watermelon (*Citrullus lanatus* var. *citroides*) seeds were planted in a glass house. Plastic pots of 25 cm (diameter) were filled with vermiculite and three seeds were planted in each pot. They were arranged in a randomized block design of 1.0 m

× 0.5 m spacing. Fresh Hoagland nutrient solution was prepared weekly and provided to the plants. Three weeks after germination, when the seedlings had developed two leaves each, two plants were removed, and the healthiest ones were retained in each pot. Pom-pom weed shoots with leaves were collected from Tzaneen, Limpopo Province, where they occur alongside the road.

2.1. Buffered Extraction

Fresh leaves and stems of the pom-pom weed were washed with distilled water, then cut into small pieces of about 0.25 cm. The phosphate buffer (pH = 7.0) was prepared for fresh extraction. A ratio of 1 g:10 ml of plant material and phosphate buffer was used for the extraction (Seddon and Schmitt, 1999). A 100 g of pom-pom weed was put into the conical flask with 1000 ml of phosphate buffer being poured into the flask. The mixture was homogenized with a homogenizer until thoroughly done, with the aim of extracting chitinase enzyme. Whatman's no.1 filter paper was used to filter homogenized mixture into another conical flask. Then, the filtrate was poured into a 1-liter bottle and stored in the refrigerator for future use.

2.2. Dry Extraction

The pompom weed leaves and stems were dried in the laboratory at ambient temperature. When completely dry, the material was ground into a powder. A 20 g dry powder was poured into a 500 ml conical flask. Then, 200 ml of 100% acetone was added into that flask. The flask was shaken on a horizontal shaker at 200 rpm for 2 hours. The mixture was filtered through Whatman's No. 1 filter paper, the residue was washed three times with 50 ml of acetone. The mixture was transferred into a pre-weighed glass petri dish and dried under a draught of air in the fume chamber overnight. Finally, the extract was dried at 60ºC for 1 hour. Thereafter, the petri dish was weighed to determine the extraction yield.

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2.3. Preparation of Homemade Fungicide

The homemade fungicide consisted of mixing 100 ml distilled water, a quarter tablespoon of baking powder, and a drop of liquid soap. To each mixture, 0.01 and 0.02 g of the dry plant extract were added to prepare, respectively 0.1 and 0.2 mg/ml plant extract fungicide.

2.4. Preparation of Commercial Fungicide

Distilled water (100 ml) was mixed with 1 ml of commercial fungicide (lime sulfur) in a 500 ml beaker, according to the manufacturer's instruction.

2.5. Treatments

Treatment started at the two-leaf stage of development. There were five treatments in triplicate, with 100 ml spraying per week. Fresh fungicide preparations were made every week just before spraying the plants. Plants were sprayed once a week for five weeks, and data were collected after week six.

Treatment A: negative control (no treatment).

Treatment B: positive control (plants were sprayed with a commercial fungicide). Treatment C: plants were sprayed with the buffered enzyme extract.

Treatment D: plants were sprayed with the acetone plant extract (0.1 mg/ml).

Treatment E: plants were sprayed with the acetone plant extract (0.2 mg/ml).

2.6. Evaluation of Efficiency

Percentage coverage of powdery mildew on leaf surfaces was determined using BioLeaf software. A camera with BioLeaf software was used to take pictures of the leaves, and then the total percentage coverage was determined. Only the upper surfaces of the leaves were measured. The efficiency of treatments was statistically analysed using one-way ANOVA (analysis of variance) using SPSS software package (ver. 25).

3. Results and Discussion

There was no sign of powdery mildew (*Podosphaera xanthii*) on plants that were sprayed with the commercial fungicide in Treatment B (0%) (Table 1). This is supported by Konstantinidou-Doitsinis (1998) who noted that chemical fungicides are effective for controlling powdery mildew. However, the plants contained residues of fungicides that persisted on the leaves (Figure 1). This, therefore, supports Fernandez-Aparicio et al. (2009) and Ali et al. (2021) who showed that chemical fungicides are now becoming of less interest as they impose residues on plant parts, which can be harmful to humans and animals. In treatment A, the plants were the most affected (77.9%), followed by plants of Treatment C (13.1%), then Treatment E (7.6%). Plants of Treatment D were the least affected (2.1%), second after plants of Treatment B.

Table 1. Number of leaves and infection percentage.

Treatments	Number of leaves	Average no. of Leaves	Infection percentage	Average infection (%)	Average infection reduction (%)
Treatment A	2	2	83.67	77,87	22,13
	2		96.67		
	2		53.28		

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Treatments	Number of leaves	Average no. of Leaves	Infection percentage	Average infection (%)	Average infection reduction (%)
Treatment B	9	8	0	0	100
	8		0		
	7		0		
Treatment C	6	4	1.17	13,1	86.9
	3		29.42		
	3		8.7		
Treatment D	8	7	2.19	2.05	97.95
	7		3.1		
	6		0.85		
Treatment E	7	6	9.43	7.56	92.44
	6		9.34		
	5		3.92		

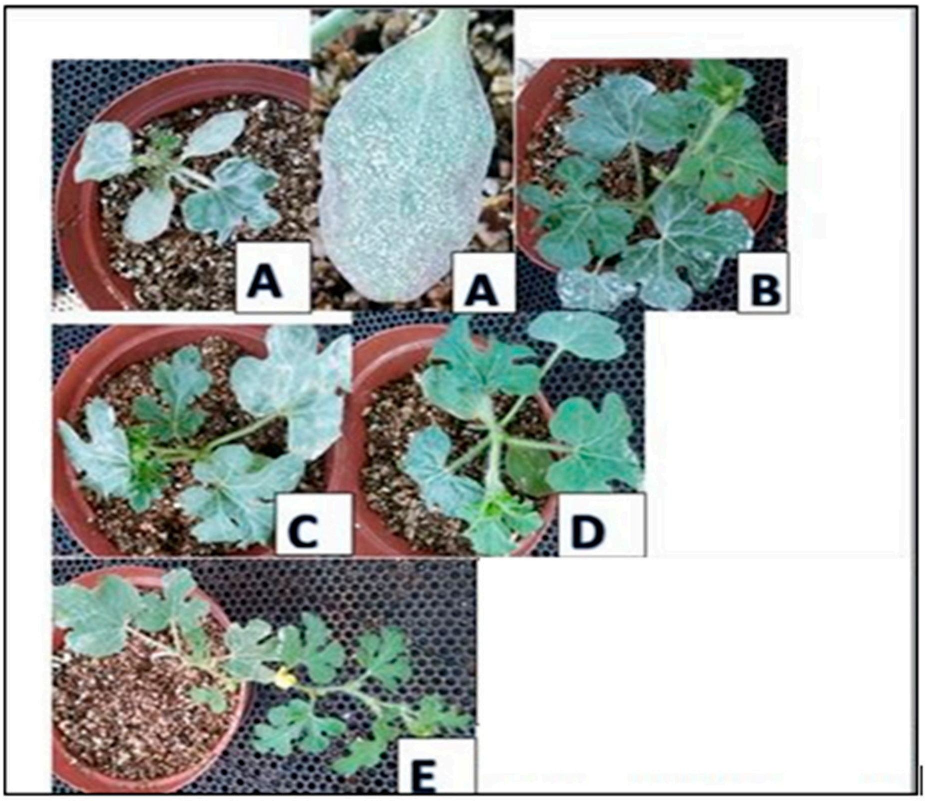

Figure 1. Plants showing rates of infections with Powdery mildew. **A**. leaves are already withering due to infestation. **B**. white spots are fungicide residues, not powdery mildew. **C-E**. Spread of infestation of powdery mildew.

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Treatment B (with no sign of powdery mildew) produced the highest average number of leaves (8) and treatment A (with the highest percentage of disease) produced the least number of leaves (2) (Figure 2). This is indicative that the higher the infection, the less the production of leaves. This might be due to the powdery mildew reducing the overall photosynthesis rate on existing leaves (Tian et al., 2024), thus decreasing the production and development of new leaves.

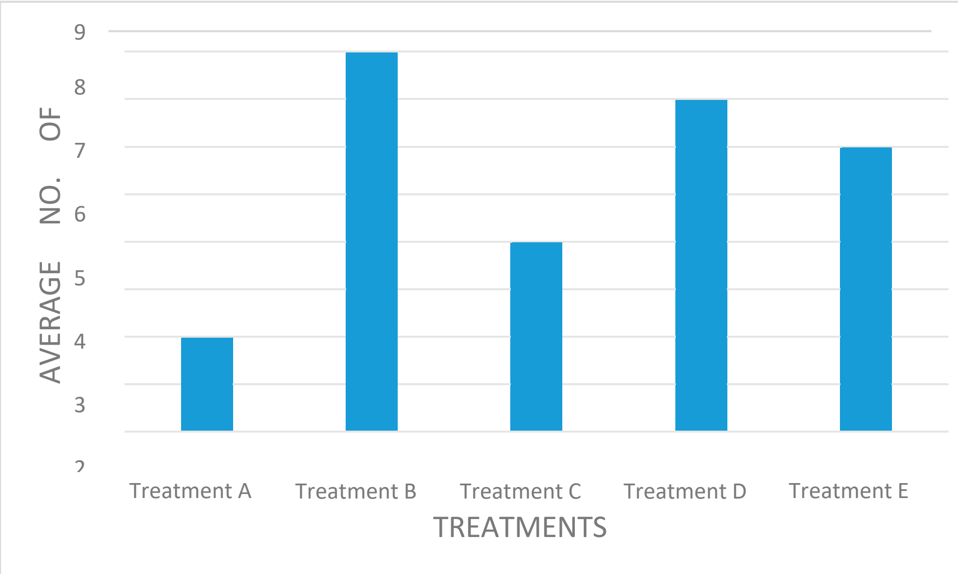

Figure 2. Average number of leaves produced per plant treatment.

One-way ANOVA showed that the five treatments were significantly different (p = 0.000). Analysis of Variance of multiple comparison showed that treatment A was significantly different to all treatments. The p-value was 0.000 for treatment A compared to the other treatments. There was no statistical difference amongst the other treatments (treatments B, C, D and E).

All treatments had an impact in limiting the growth and spread of powdery mildew (Figure 3). The dry extract treatments (Treatments B and D) were the most effective fungicide. Treatment C (buffered extract) had a lower effect, which can be attributed to the fact that the plant material was not ground, only cut into smaller pieces. Eloff (1998) showed that there was a higher rate of extraction from particles that were very fine than those acquired after 24h in a shaking machine with only a small amount of fine ground material. Therefore, plant material has to be ground to powder form, whether dry or wet, to increase the surface area to extract antimicrobial compounds and therefore increasing the extraction rate. Therefore, the ability of pom-pom weed extracts to control powdery mildew can be due to the production of one or more of these secondary metabolites (observed from the acetone extract treatments) and the ability to produce chitinase enzyme (observed from the buffered extract treatment).

The results obtained for treatment E are in concord with the ones acquired by Seddon and Schmitt (1999) whereby they used medicinal plants to control powdery mildew on squash plants. This is due to the phytochemical properties available in the plant extract (McGaw et al., 2022). However, baking powder alone was considered not effective in controlling powdery mildew and needs to be mixed with neem oil (Otten, 1997). Our study shows that pompom weed extracts are effective in controlling powdery mildew as neem oil was omitted in the homemade fungicide's ingredients of the study.

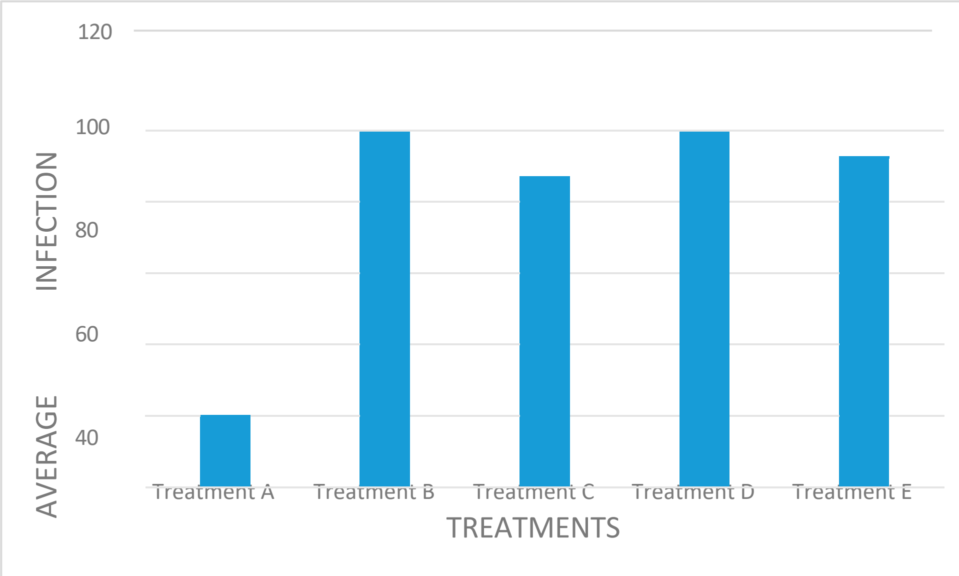

Figure 3. Average powdery mildew infection reduction.

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Data availability statement: The authors verify that the data from which the study's conclusions are based is available.

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