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Efficacy of salicylic and oxalic acids against *Phytophthora*-induced hollow stem rot of cabbage in Egypt

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Abstract
Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important and widely cultivated winter cruciferous vegetables in Egypt. Six fungal isolates were obtained from rotted tissues of cabbage hollow stems and identified based on morphological characteristics: *Phytophthora dreschleri* (3 isolates), *Fusarium oxysporum* (1), *F. verticillioides* (1), and *Alternaria alternata* (1). Pathogenicity tests revealed that *Phytophthora* sp. successfully infected wounded cabbage stems, producing large rotting cavities with disease incidences of 60.2%, 40.1%, and 10.3%, whereas the other isolates were non-pathogenic. The antifungal efficacy of salicylic acid (SA) and oxalic acid (OA) was evaluated *in vitro* and in greenhouse conditions. OA at 30% inhibited mycelial growth by 94% and reduced disease severity by 85%, while SA at 10% inhibited mycelial growth by 96% and reduced disease severity by 82% compared to the control. These findings suggest that both SA and OA can serve as effective, eco-friendly inhibitors of cabbage hollow stem rot under Egyptian conditions.

Keywords: Cabbage, hollow stem rot, *Phytophthora* sp., salicylic acid, oxalic acid

1. Introduction

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important cruciferous leafy vegetables cultivated worldwide. In Africa, Egypt ranks among the leading producers, together with South Africa, Rwanda, Ethiopia, Kenya, and Angola [1]. During 2013-2014, Egypt cultivated approximately 40,965 hectares of cabbage, yielding 515,749 tons at an average of 12.59 t/ha [2]. By 2023, the cultivation area declined to 17,406 hectares; however, production reached 508,572 tons, with an increased average yield of 29.22 t/ha [3].

Despite its economic significance, cabbage production is constrained by several physiological and pathological disorders. Among these, hollow stem disorder is particularly damaging in Brassica crops. It is often associated with nutrient imbalances, especially calcium and boron deficiencies or excessive nitrogen supply [4]. The disorder begins with the development of small elliptical fissures in the internal stem tissues, which gradually expand into cavities. In severe cases, the hollowing extends throughout the stem and into the cabbage head, greatly reducing marketability. These cavities frequently serve as entry points for secondary infections, leading to internal decay and discoloration. Pathogen invasion typically initiates from the lower stem and roots, with the mycelium progressing upward through the xylem vessels. As the vessels become blocked, water and nutrient transport is disrupted, ultimately weakening the plant.

In recent years, plant defense inducers have emerged as promising tools for the sustainable management of crop diseases. The efficacy of such compounds is often influenced by their method of application, with trunk injection and root/soil application generally outperforming foliar sprays [5-9]. Systemic acquired resistance (SAR) inducers, including acibenzolar-S-methyl, salicylic acid, oxalic acid, and potassium phosphates, have been reported to significantly reduce disease severity in several patho systems. For instance, trunk injection of these compounds suppressed Huanglongbing (HLB) disease progression and reduced *Candidatus Liberibacter asiaticus* titers under field conditions [10]. Similarly, trunk injection has improved the effectiveness of control agents against fire blight, offering an alternative to antibiotics in open environments [11].

The present study was undertaken to isolate and identify fungal pathogens associated with hollow stem rot in cabbage under Egyptian conditions and to evaluate the antifungal efficacy of oxalic acid and salicylic acid *in vitro* and *in vivo*.

2. Materials and Methods

2.1 Sample collection

Four symptomatic cabbage plants showing hollow stem rot were collected from different localities in Assiut Governorate, Egypt (Manfalout, El-Hawatka, Reefa, and Shatb).

2.2 Pathogen isolation

Infected stem tissues (1-2 mm^2) were excised from the rotted areas, surface-sterilized in 5% sodium hypochlorite for 3 min, rinsed three times with sterile distilled water, and blot-dried on sterile filter paper. The disinfected tissues were placed on potato dextrose agar (PDA) plates and incubated at 25 °C. After 5 days, emerging colonies were purified and maintained on PDA for further study.

Fig 1: Symptoms of hollow stem rot in cabbage.

2.3 Pathogenicity tests

Pathogenicity assays were conducted in July 2022 in the greenhouse of the Plant Pathology Department, Faculty of Agriculture, Assiut University. Spore suspensions (2×10^5 cfu/ml) prepared from 7-day-old colonies were used to inoculate two-month-old cabbage seedlings. The soil around the stem base was infested and covered with Parafilm to maintain moisture. Non-inoculated seedlings served as controls. Each treatment was replicated three times with two plants per replicate. Disease incidence was assessed to confirm pathogenicity [12].

2.4 *In vitro* evaluation of salicylic and oxalic acids

The antifungal effects of salicylic acid (SA; 99% purity, Sigma-Aldrich) and oxalic acid (OA; ≥99% purity, Sigma-Aldrich, St. Louis, MO, USA) were tested against *Phytophthora* sp. For SA, concentrations of 2%, 5%, and 10% were used, while OA was applied at 10%, 20%, and 30%. For each treatment, 90 ml of PDA was amended with 10 ml of the compound solution, gently rotated to ensure even distribution, and allowed to solidify. Mycelial discs of *Phytophthora* sp. were placed at the center of the plates, which were incubated at 25 °C for 7 days. Control plates contained the pathogen without any treatment. Colony diameters were measured, and growth inhibition was calculated using the formula of [13]:

$$\text{Inhibition (\%)} = \left(\frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}}\right) \times 100$$

2.5 Greenhouse experiment

A greenhouse trial was conducted in July 2023. Two-month-old cabbage seedlings were inoculated with *Phytophthora* sp. (2×10^5 cfu/ml) by soil infestation. After two weeks, infected plants were treated with either 30% OA or 10% SA. Soil around the stem was covered with Parafilm to maintain humidity. Plants inoculated with the pathogen alone served as controls. Disease incidence and inhibition percentage were determined using the same formula [13].

2.6 Statistical analysis

All experiments were arranged in a completely randomized design (CRD). Data were expressed as mean ± standard error (SE). Analysis of variance (ANOVA) was performed using [software, e.g., CoStat, SAS, or SPSS], and treatment means were compared using Duncan's multiple range test (DMRT) at $p \leq 0.05$ to determine significant differences.

3. Results and Discussion

3.1 Fungal isolation and identification

Diseased cabbage plants showing hollow stem rot were collected, and six fungal isolates were obtained. Based on morphological characteristics, the isolates were identified as *Phytophthora dreschleri* (three isolates), *Fusarium oxysporum* (one isolate), *F. verticillioides* (one isolate), and *Alternaria alternata* (one isolate). Previous studies reported that *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phytophthora* are common pathogens associated with root and basal rot of cabbage in Egypt [14], supporting the present findings.

3.2 Pathogenicity test

Two weeks after inoculation with a 2×10^5 cfu/ml spore suspension, typical hollow stem symptoms were observed in inoculated plants. Initial symptoms included small fissures in the inner stem tissue, which gradually expanded into cavities and large rotting lesions (Figure 2). Among the tested isolates, *Phytophthora* sp. produced the highest disease severity, with incidences of 60.2%, 40.1%, and 10.3% across the three isolates, whereas *A. alternata*, *F. oxysporum*, and *F. verticillioides* were non-pathogenic (Table 1).

These results are consistent with reports of *Phytophthora* spp. causing root rot in cabbage and cauliflower in Oregon [15] and South Africa [16], as well as stem rot of lettuce in Greece [17]. Other species of *Phytophthora* have also been implicated in crop losses, such as *P. cinnamomi* in pineapple [18] and *P. nicotianae* in Washingtonia palms [19]. Although *A. alternata* is widely known to cause heart rot in pomegranate [20], it was not pathogenic on cabbage in the current study.

Fig 2: Pathogenicity test of cabbage

Table 1: Pathogenic capability of fungal isolates associated with hollow stem rot in cabbage.

Isolated fungi	Mean% Disease severity
Phytophthora sp. 1	60.2
Phytophthora sp. 2	40.1
Phytophthora sp. 3	10.3
Fusarium oxysporum	0.0
F. verticillioides	0.0
Alternaria alternata	0.0

3.3 *In vitro* evaluation of salicylic and oxalic acids

Different concentrations of SA and OA significantly inhibited the mycelial growth of *Phytophthora* sp. (Table 2; Figures 3-5). The strongest effect of OA was observed at 30%, which reduced mycelial growth by 94%. Similarly, SA at 5% and 10% inhibited growth by 92% and 96%, respectively.

The antifungal activity of SA and OA may be linked to their roles as defense inducers, triggering phytoalexin production, enhancing protein and carbohydrate metabolism, and increasing photosynthetic pigments in treated plants [21-24]. Comparable findings were reported in tomato, where SA (30 mg/l) effectively suppressed early blight caused by *Alternaria solani* [25]. Similarly, OA was identified as a major active compound in waste effluent-derived soil management solutions (WESMS), reducing *Phytophthora* blight in pepper at concentrations as low as 200 mg/l [26].

Table 2: Effect of salicylic and oxalic acids on mycelial growth of Phytophthora sp. *in vitro*

Treatment	Inhibition of mycelial growth (%)
OA 10%	60
OA 20%	80
OA 30%	94
SA 2%	88
SA 5%	92
SA 10%	96

LSD ≤ 0.05 = 3.8

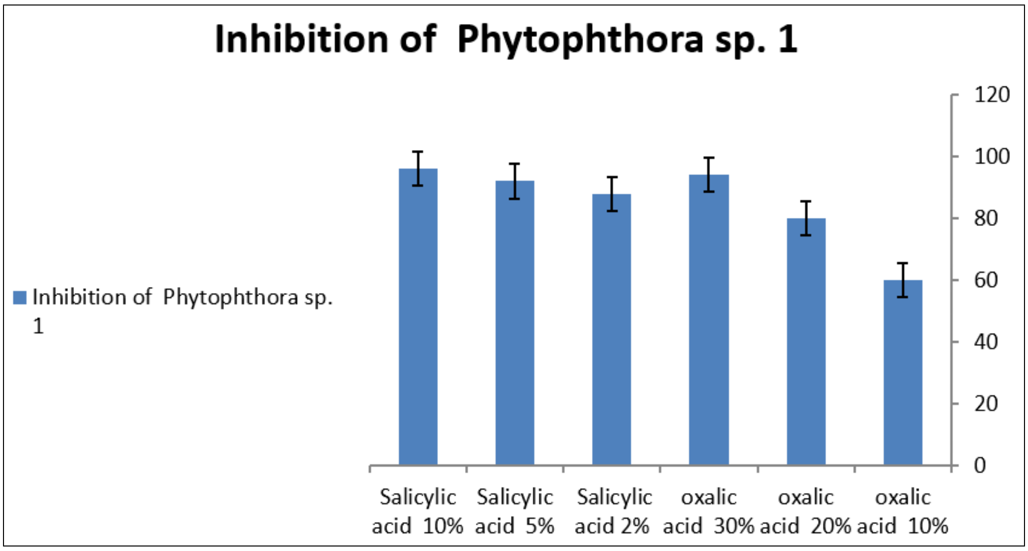

Fig 3: Impact of varying oxalic and salicylic acid concentrations on *phytophthora sp*. mycelial development *in vitro*

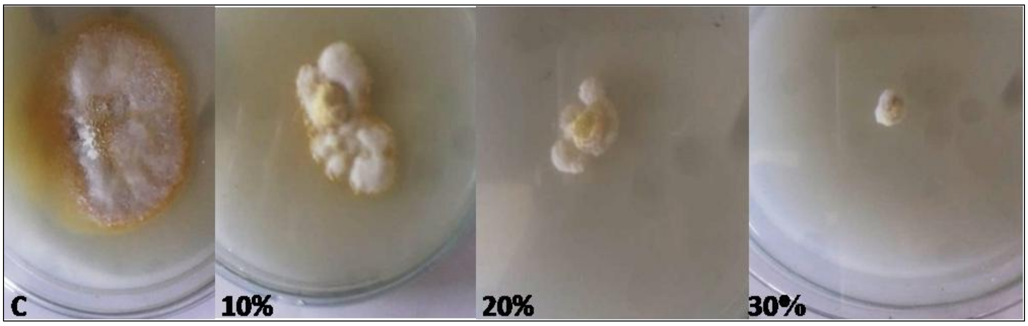

Fig 4: showing effect of diffierent concentrations of oxalic acid on myecelial growth of *phytophthora* sp.

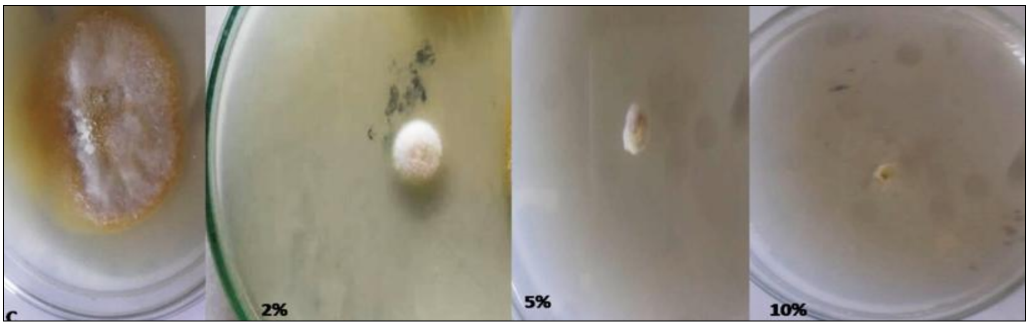

Fig 5: Impact of varying salicylic acid concentrations on *phytophthora sp.* myecelial growth.

3.4 Greenhouse evaluation

Under greenhouse conditions, both SA and OA significantly reduced disease severity caused by *Phytophthora* sp. (Table 3; Figures 6-7). The highest reduction was observed with 30% OA, which decreased disease severity by 85% compared to the untreated control. Salicylic acid was also effective, reducing disease severity by 75% at 5% and 82% at 10%.

These findings confirm the potential of SA and OA as alternative management tools against cabbage hollow stem rot. Comparable disease reductions were observed in pineapple hybrids, where high-volume applications suppressed *Phytophthora*-induced root and heart rot [18]. Similarly, soil mixing and root dipping treatments have been reported to control Pythium rot in Chinese cabbage [14]. Application methods such as trunk injection, successfully used in fire blight control [11], may further improve the delivery and efficacy of such resistance inducers.

Table 3: Effect of salicylic and oxalic acids on disease severity of *Phytophthora* sp. *in vivo*

Treatment	Reduction of disease severity (%)
OA 30%	85
SA 5%	75
SA 10%	82
Healthy control	100
Infected control	0

LSD ≤ 0.05 = 6.2

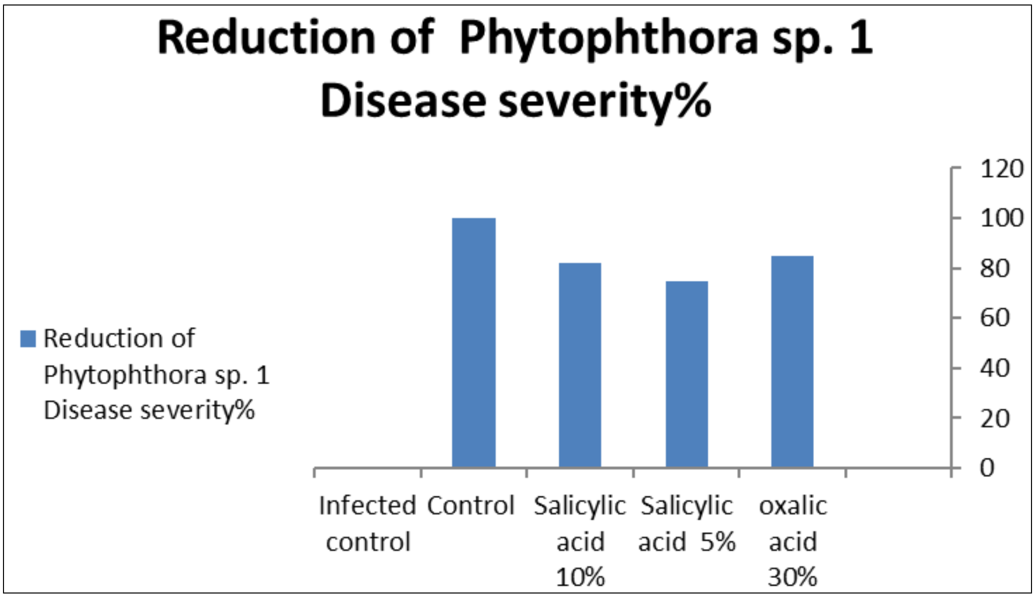

Fig 6: Impact of varying oxalic and salicylic acid concentrations on *phytophthora sp. in vivo*

Fig 7: C: Treatment of the hollow stem with *phytophthora* sp. only (show rotting), TO1,2,3: treatment the hollow stem of cabbage with 30% conc of oxalic acid (no rotting tissue) and TS 1,2,3 treatment the hollow stem of cabbage with 10% conc of salicylic acid (no rotting tissue).

3.5 Conclusion

The present study identified *Phytophthora dreschleri* as the primary causal agent of hollow stem rot in cabbage under Egyptian conditions. Both salicylic and oxalic acids demonstrated strong antifungal activity *in vitro* and *in vivo*. Specifically, OA at 30% reduced mycelial growth by 94% and disease severity by 85%, while SA at 10% reduced growth by 96% and disease severity by 82%. These results suggest that SA and OA can be integrated into disease management strategies as eco-friendly alternatives for controlling cabbage hollow stem rot in Egypt.

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