ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

# Differential Susceptibility of Bean Pods, Bell Pepper Fruits, and Soybean Leaves Inoculated With *Colletotrichum Capsici* Enzymes

**Jolina A. Enardecido*[1,2], Maricel J. Didal[1,3], Ismael T. Cabalinan[1,3], and Merlina H. Juruena[1]**

**[1]University of Southeastern Philippines, Tagum-Mabini Campus, Tagum City, 8100, Philippines**

**[2]Monkayo College of Arts, Sciences, and Technology, Monkayo, Davao de Oro, 8805, Philippines**

**[3]Department of Agriculture, Regional Crop Protection Center, Trento, Agusan del Sur, 8505, Philippines**

***Corresponding Author**

**DOI: https://doi.org/10.51244/IJRSI.2025.120800352**

**Received: 05 Sep 2025; Accepted: 12 Sep 2025; Published: 14 October 2025**

## ABSTRACT

*Colletotrichum* spp. causes anthracnose diseases in various crops by producing cell wall-degrading enzymes (CWDE's). Although these enzymes are known to play a role in pathogenesis, their direct effects on host tissues and contribution to disease development remain poorly understood. In this study, *Colletotrichum capsici* was isolated from bell pepper, and its enzymatic activities were tested on bean pods, bell pepper fruits, and soybean leaves to determine the role of enzymes in disease development. The study focused on characterizing the effects of enzymatic tissue degradation based on symptom appearance, disease incidence percentage, and the infection severity level. Pathogen discs (10 mm) were cultured in Potato Dextrose Broth (PDB), and crude filtrates (CF) were obtained through sequential filtration. Plant tissues were inoculated with CF concentrations of 25%, 50%, 75%, and 100%, arranged in a Completely Randomized Design (CRD), and analyzed using analysis of variance (ANOVA). Results showed that symptom expression occurred as early as 12 hours post-inoculation. At 100% CF, symptoms appeared at 1.75, 2.75, and 0.50 days in bean pods, bell pepper fruits, and soybean leaves, respectively. Disease incidence reached 93.75%, 58.33%, and 100%, while severity reached 26.25%, 21.67%, and 68.33% in bean pods, bell pepper fruits, and soybean leaves, respectively. The study demonstrated that higher CF concentrations consistently accelerated symptom onset and increased both disease incidence and severity. These results confirm that the pathogenicity of C. capsici is closely associated with its enzymatic activity, underscoring the critical role of fungal enzymes in host tissue degradation. This knowledge provides valuable insights for resistance breeding and the development of enzyme-targeted disease management strategies.

**Keywords:** Colletotrichum, anthracnose disease, enzymes, crude filtrate, tissue degradation

## INTRODUCTION

*Colletotrichum* spp. are among the major fungal plant pathogens responsible for anthracnose diseases, which affects a wide range of hosts (Freeman et al., 1998), including peppers (*Capsicum* spp.). These pathogens can infect multiple plant organs, including roots, stems, leaves, and fruits. Most crops grown worldwide are susceptible to one or more *Colletotrichum* species (Dowling et al., 2020), making anthracnose a significant global economic constraint to crop production. The disease symptoms are typically characterized by sunken lesion of varying colors on leaves, stems, fruits, or flowers. These lesions often enlarge, usually leads to wilting, withering, and eventual death of infected plant tissues (Mehbub et al., 2006). The pathogen is cosmopolitan in distribution, with primary inoculum disseminated by wind or rain (Ayaa, 2021). During host invasion, *Colletotrichum* spp. employ diverse strategies, ranging from intracellular hemibiotrophy to subcuticular intramural necrotrophy (Jeyaraj et al., 2023). Moreover, they develop specialized infection structures, including germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Enzymes play a critical role in the pathogenicity of *Colletotrichum* spp. The pathogen produces an arrays of cell wall-degrading enzymes (CWDEs), such as cellulases, hemicelluloses, pectinases, and proteases, which breakdown the plant cell wall and facilitate fungal penetration into host tissues (Thaker, 2022). Similarly, Zhang et. (2022), stated that the pathogen produces cutinases and lipases to breach the cuticle of the host plant, degrading the waxy cuticle, and thereby allowing the fungus to access the plant tissue. Moreover, *Colletotrichum* spp. secretes enzymes involved in the degradation of complex plant polymers, such as ligninases and xylanases, to degrade complex polymers of the host tissues (Kachroo, 2022). During colonization of host tissues, *Colletotrichum* spp. can modify the extracellular matrix of the host plant by secreting enzymes such as glucanases and chitinase, which facilitates hyphal growth and dissemination within the host (Riseh et al., 2024).

Among the *Colletotrichum* species, *C. capsici* is one of the most frequently reported fungal plant pathogens in the Philippines (Balendres, 2023). It is a filamentous phytopathogenic fungus, and it secretes several enzymes during the infection process, primarily targeting the host plant cell wall as part of its initial infection activity, leading to anthracnose disease (Gu et al., 2024). In this study, *Colletotrichum capsici* was isolated to determine the effects of the different concentration level of crude filtrate (CF) containing enzymes on disease development. Inoculated tissues of bean pods, bell pepper fruits, and soybean leaves were evaluated for the symptom appearance, disease incidence, and severity of infection. Understanding fungal enzymatic activity in disease development is essential for advancing knowledge of plant-fungal enzymes interactions and developing effective disease management.

## MATERIALS AND METHODS

### Preparation of Culture Medium

The commercial Potato Dextrose Agar (PDA) medium was prepared following the procedure in the label using 39g PDA powder per liter of distilled water. The PDA was sterilized at 15 psi for 20 minutes. This was conducted at the PCR Laboratory of the University of Southeastern Philippines, Tagum- Mabini Campus, Apokon, Tagum City, Philippines.

### Isolation of the Pathogen

The fungus was isolated from infected bell pepper fruits showing symptoms of anthracnose disease from the nearby public market. The fruits were washed with tap water, and small sections (3-6 mm long) of the tissue with lesions were cut off and dipped into previously sterilized petri plates containing 1% sodium hypochlorite (NaOCl) for one minute, then rinsed thrice with sterile distilled water (SDW) following the method of Peraza-Sanchez et al., (2005). The disinfected tissue was isolated using the PDA medium and incubated at room temperature. A microscopic examination was done to confirm the identity of the fungus based on morphology. Pure culture of *C. capsici* was characterized by a slow to medium growth, and have olive brown to pinking colony. The aerial mycelium was soft, sticky, and becoming flat on the upper surface. Conidiophores were formed as short single phiallides on hyphae. Spores were whitish and elongated.

### Preparation of Broth Medium

Potato Dextrose Broth (PDB) was prepared following the procedure of Yokota et al., (2010) using 24g of commercial PDB added with 1 liter of distilled water and boiled. The broth was filtered using gauze cloth and supplemented with 10g of dextrose powder. The mixture was transferred to a 250ml Erlenmeyer flask and sterilized at 15 psi for 20 minutes.

### Preparation of Fungal Enzyme

Seven-day old *Colletotrichum capsici* pure culture was utilized for fungal enzyme production. Agar bits of *C. capsici* culture were inoculated and allowed to grow in the sterilized PDB medium. After 7 days, the fungal mycelia were removed from the medium by filtering, using 3 layers of gauze cloth above and 3 layers of filter paper below, this will allow the filtrate to drain and then it was centrifuged to separate the supernatant from the

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

pellet. The filtrate (supernatant) was collected using a syringe microfilter (0.2um) and used in the tissue maceration experiment to retain the broth with enzymes. First filtration used triple layered cheesecloth, second filtration used 3 layered filter paper, and third filtration used a syringe filter. Then, the presence of spores was microscopically examined to ensure there were no single spores in the filtrate. This was conducted at the Regional Crop Protection Center (RCPC), DA-Caraga Region.

### Inoculation of Fungal Enzyme in Filtrate

After a week of fungal cultivation in PDB, each plant tissues were inoculated with different concentrations of culture filtrate (CF) of *C. capsici* by drenching the suspension. Test plant tissues were monitored for a week until symptoms appeared. The number of days from inoculation of the fungal enzyme to the first appearance of symptoms was counted and recorded. The incidence was evaluated and recorded regularly until the termination of the study with the formula %Disease Incidence = No. infected tissue/Total no, of tissue assessed x 100. The severity of the disease was assessed using the arbitrary rating scale of Miller-Butler et al., (2019) with slight modifications, where 0-no disease infection, 1- very slight (1-20%) infection symptoms, 2- slight (21-40%) infection symptoms, 3- moderate (41-60%) infection symptoms, 4- moderately heavy (61-80%) infection symptoms, 5-heavy (81-100%) infection symptoms. And based on the standard rating scale of Mohamed et al., (2000), degree of infection (% Disease Index) was computed as % Disease Index = $0n^0+1n^1+2n^2+3n^3+4n^4+5n^5$ / (maximum grade) total population x 100, where: $0n^0+1n^1$…. $+5n^5$ refers to the number of samples showing the rating scale of 0,1,2,3,4, and 5.

### Statistical Analysis

This experiment was laid out in a Completely Randomized Design (CRD) with five treatments replicated four times in varying concentration level of crude filtrate (CF): $T_1$-Untreated, $T_2$-25% CF, $T_3$-50% CF, $T_4$-75% CF, $T_5$-100% CF. Data collected was analyzed using the analysis of variance, and further test were done using Tukey's Honest Significant Difference (THSD).

### Ethical Considerations

The study was conducted in a controlled laboratory setting, and all experiments were designed to minimize waste and prevent environmental harm. The researchers ensured that all chemicals and materials used in the study were handled and disposed in accordance with local regulations and guidelines. By acknowledging and addressing these ethical considerations, the researchers aimed to ensure the integrity of the research process and contribute to the development of responsible and sustainable agricultural practices.

## RESULTS AND DISCUSSION

### Days of Symptom Appearance

Bean pods, bell pepper fruits, soybean leaves exhibited symptoms within 120 hours of observation. In bean pods, the earliest symptoms were observed at 100% crude filtrate (CF), with an average of 1.75 days after inoculation, while the latest symptoms appeared at 25% CF, averaging 3.88 days after inoculation. These results suggest that higher concentrations of fungal enzymes accelerated symptom development. In bell pepper fruits, symptoms appeared at 2.75 days with 100% and 75% CF, whereas 50% CF resulted in symptom appearance after 4 days, and 25% CF produced no symptoms. Although statistical analysis revealed no significant differences among treatments, the trend indicated that higher CF concentrations reduced the time to symptom appearance. A similar pattern was observed in soybean leaves, where 100% CF induced symptoms at 0.50 days, while 25% CF delayed symptom expression to 2.01 days. Again, no significant differences were detected among treatments (Table 1). Overall, fungal enzymes in the CF influenced the rate of symptom development differently depending on host tissue and enzyme concentration. In general, higher CF concentrations tended to accelerate symptom appearance across all hosts, with soybean leaves displaying the highest sensitivity, showing symptoms earlier and at lower concentrations compared to bean pods and bell pepper fruits.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Table 1. Mean number of days of symptom appearance after inoculation of fungal enzymes in the crude filtrate on PDB filtrate to bean pods, bell pepper fruits, and soybean leaves

| TRT | Bean pods | Bell pepper fruits | Soybean leaves |
|---|---|---|---|
| Control | none | none | none |
| 25 % CF | $3.88^{b}$ | none | $2.01^{ns}$ |
| 50 % CF | $3.06^{b}$ | $4.00^{b}$ | $2.00^{ns}$ |
| 75 % CF | $3.01^{b}$ | $2.75^{a}$ | $1.58^{ns}$ |
| 100 % CF | $1.75^{a}$ | $2.75^{a}$ | $0.50^{ns}$ |

Values are the means of each treatment with four replications; means of the same superscript are not significantly different; $^{ns}$ means not significant; and none means no symptoms.

The number of days to cause symptoms inoculated with enzymes of *C. capsici* differs depending on several factors such what enzyme is present, the genetic make-up of the host plant, and the environmental conditions (Wijesundera et al. 1989). Enzymatic tissue degradation activity of *C. capsici* relative to symptom appearance totally occurs within 5-7 days of post-inoculation. As for bean pods and soybean, similar trend was observed from the study of Prajapati et al., (2014) in which symptom appeared around 4-6 days of post-inoculation of pathogen filtrate. Another study from Silva et al., (2021), the infection symptoms of tomato inoculated with the *Colletotrichum coccodes* enzymes, appeared before 7 days after inoculation, mainly in fruits inoculated with artificial wound. The morphology of the different plant organs differs, thus soybean leaves with larger, and have higher number of stomates where the mean stomatal frequency on the adaxial surface was 130; on the abaxial surface, it was 316 stomata/$mm^2$ (Ciha & Brun, 1975), was infected early as compared to bean pods, and bell pepper fruits. This phenomenon implied that enzymatic tissue degradation activities in the pathogenicity of *C. capsici* is crucial to understand for the disease management establishment.

**Disease Incidence (%)**

Percentage of disease incidence was evaluated to assess the extent and severity of disease within the population of the specific crop. Table 2 shows the means of the disease incidence percentage on bean pods after 12, 24, 48, 72, 96 and 120 hours of inoculation of fungal enzyme in the crude filtrate. Results revealed that 50%, 75%, and 100% CF exhibited disease incidence on the 48$^{th}$ hour after inoculation with 12.50%, 18.75% and 75%, respectively. Tissues inoculated with 25% exhibited disease incidence on the 72$^{nd}$ hour after inoculation with 6.25% while other treatments progressed rapidly. Disease incidence progressed until 120$^{th}$ hour after inoculation with 100% CF bearing the highest disease incidence of 93.75%. It was followed by 75% CF, 50% CF and 25% CF with 81.25%, 68.75% and 62.50%, respectively. Statistically, results of disease incidence on bean pods bear significant difference among treatments. More so, it was observed that, higher concentrations of fungal enzymes result in higher disease incidence percentages

Table 2. Means of the disease incidence percentage after 120 hours of inoculation of fungal enzyme in crude filtrate on bean pods

| Treatments | Hours of Incubation | | | | | |
|---|---|---|---|---|---|---|
| | 12h | 24h | 48h | 72h | 96h | 120h |
| T1-control | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{b}$ |
| T2-25% | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $6.25^{c}$ | $43.75^{b}$ | $62.50^{a}$ |
| T3-50% | $0.00^{ns}$ | $0.00^{ns}$ | $12.50^{b}$ | $56.25^{b}$ | $68.75^{ab}$ | $68.75^{a}$ |

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

| | | | | | | |
|---|---|---|---|---|---|---|
| T4-75% | $0.00^{ns}$ | $0.00^{ns}$ | $18.75^{b}$ | $50.00^{b}$ | $75.00^{ab}$ | $81.25^{a}$ |
| T5-100% | $0.00^{ns}$ | $0.00^{ns}$ | $75.00^{a}$ | $93.75^{a}$ | $93.75^{a}$ | $93.75^{a}$ |
| Pr (> F) | - | - | 0.0046 | 0.0000 | 0.0014 | 0.0002 |

Values are means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ – not significant

Similar trend was also observed in bell pepper fruit as presented in Table 3 which revealed that crude filtrate with fungal enzymes inoculated on bell pepper fruits, resulted to no disease incidence in all treatment during the first 48 hours after inoculation. 75% and 100% CF exhibited disease incidence on the 72$^{nd}$ hour after inoculation with 41.67% and 58.33%, respectively, with no progress on the next succeeding period. 50% CF exhibited disease incidence on the 96$^{th}$ hour with 8.33%, and 25% CF did not exhibit disease incidence at all. Statistically, results revealed significant difference among treatments only after 120 hours of observation. Results further revealed that, higher concentrations of unboiled fungal enzymes result in higher disease incidence percentages in bell pepper. Meanwhile, in soybean leaves, Table 4 showed that 50%, 75% and 100% CF exhibited 8.33%, 75% and 100% disease incidence after 12 hours of inoculation, respectively. 25% CF exhibited disease incidence after 48 hours with initial rate of 58.33%. After 120 hours, 50%, 75% and 100% CF exhibited 100% disease incidence while 25% CF remained at 66.67%. Statistically, inoculation with lower concentration (25%) CF has significant difference among concentration treatments.

Table 3. Means of the disease incidence percentage after 120 hours of inoculation of fungal enzymes in crude filtrate inoculated on bell pepper

| **Treatments** | **Hours of Incubation** | | | | | |
|---|---|---|---|---|---|---|
| | **12h** | **24h** | **48h** | **72h** | **96h** | **120h** |
| T1-control | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ |
| T2-25% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ |
| T3-50% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $8.33^{bc}$ | $8.33^{bc}$ |
| T4-75% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $41.67^{a}$ | $41.67^{ab}$ | $41.67^{ab}$ |
| T5-100% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $58.33^{a}$ | $58.33^{a}$ | $58.33^{a}$ |
| Pr (> F) | - | - | - | 0.0056 | 0.0119 | 0.0119 |

Values are means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ means not significant

Study of Prajapati et al., (2014), discusses how the extracellular glucoamylase of *Colletotrichum* sp. KCP1 produced by solid state fermentation, and purified by ammonium sulphate and gel permeation chromatography, resulted to the stability of the enzymes over wide pH range and at 40-50$^{0}$C temperature at 120 minutes. The varied cellular temperature and pH of the different plant tissues influenced the enzymatic activity of the pathogen relative to tissue degradation where enzymes exhibited maximum activity at 50$^{0}$C with pH 5.0 (Prajapati et al., 2014). Additionally, fungal plant pathogens release various extracellular enzymes to degrade cell wall polymers from plants to obtain nutrients and ensure infection during invasion process of plant tissues (Kubicek et al., 2014).

*Colletotrichum* spp. is known as an enzyme producing pathogen during infection processes especially in tropical and subtropical fruits such as the high valued crops as mango, strawberry, avocado, citrus, papaya, cashew, and passion fruits (Lakshmi et al., 2011), thus the symptom development with the application of the

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

crude filtrate of *Colletotrichum* spp. infected the crops in this study, and the symptoms was prevalent. Moreover, the fungal pathogen is reported to produce cutinases, allowing the pathogen to penetrate through the cuticle of the host plant. In addition, *Colletotrichum* spp. also produces pectate lyase, proteinases, lipases, cellulases, amylases, and esterases (Martinez and Maicas, 2021).

Table 4. Means of the disease incidence percentage after 120 hours of inoculation of fungal enzyme in the crude filtrate on soybean leaves

| **Treatments** | **Hours of Incubation** | | | | | |
|---|---|---|---|---|---|---|
| | **12h** | **24h** | **48h** | **72h** | **96h** | **120h** |
| T1-control | $0.00^{b}$ | $0.00^{c}$ | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ |
| T2-25% CF | $0.00^{b}$ | $0.00^{c}$ | $58.33^{a}$ | $66.67^{b}$ | $66.67^{b}$ | $66.67^{b}$ |
| T3-50% CF | $8.33^{b}$ | $58.33^{b}$ | $91.67^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| T4-75% CF | $75.00^{a}$ | $83.33^{ab}$ | $83.33^{a}$ | $91.67^{ab}$ | $100.00^{a}$ | $100.00^{a}$ |
| T5-100% CF | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ | $100.00^{a}$ |
| **Pr (> F)** | **0.0000** | **0.0000** | **0.0009** | **0.0000** | **0.0000** | **0.0000** |

Values are means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ means not significant

Bell pepper fruits resulted to lower disease incidence as compared to beans and soybean due to the *CaChiIII7* chitinase gene that has been identified and isolated from pepper plants by Ali et al., (2020), where the transient expression of *CaChiIII7* gene in pepper increases the basal resistance to *C. acutatum* by significantly expressing several defense response genes and the hypersensitive response (HR), accompanied by an induction of $H_2O_2$ biosynthesis. As for the bean pods, lower disease incidence percentage can be influenced by the proteinaceous inhibitors of the enzymes released by the *C. capsici* as discussed by Wijesundera et al., (1989), where they observed that Polygalacturonase produced by *Colletotrichum lindemuthianum* race γ have no activity detected after inoculation. Whereas, the 100% incidence in soybean leaves were entirely due to increase photosynthetic activity contributing to increase energy usage inhibiting the establishment of defense mechanism, and the tissue morphology where there are larger and hinger number of stomates (Ciha & Brun, 1975), thereby contributing to the rapid infection, as the pathogen developed and produces a wide array of enzymes and toxins contributing to successful pathogenesis (Pring et al., 1995).

**Disease Severity (%)**

On bean pods (Figure 1), it was observed that on 12 and 24 hours after inoculation, results revealed that 50%, 75%, and 100% CF exhibited infection with 2.50%, 2.50%, and 15% severity, respectively (Table 5). 25% CF exhibited disease severity only after 72 hours while other CF concentration progressed rapidly. Slower progress was noted on 50% CF and 100% CF after 96 hours with the severity rate of 13.75 and 21.25% from previous 11.25 and 20% respectively, while rapid progress was observed on 25% and 75% CF with 8.75 and 16.25% from previous 1.25 and 8.75%, respectively. After 120 hours of CF inoculation, slow progress was observed on 25%, 75%, and 100% CF with 12.50%, 18.75%, and 26.25%, respectively, while samples applied with 50% CF remained at 13.75%. Statistical analysis revealed that 100% CF has no significant difference to 75% CF but differed significantly to the rest of the treatments, while 25% CF and 50% CF and Treatment showed no significant difference with each other.

Bean plants has cuticle as a protective outer layer to pathogen attack, containing proteins such as pectin, cellulose, and hemicelluloses that can be a substrate for the enzymes produced by *Colletotrichum capsici* (Huang, 2013). These enzymes degrade the cell wall components of bean, thus allowing the fungus to

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

penetrate inside its host's tissues (Pring et al., 1995). During the infection process of *C. capsici*, its spores may land on the bean pod surface, then germ tube and appressoria may develop facilitate penetration into the cuticle and cell wall. Enzymes such as the pectinases and cellulases breaks down those cell wall components. Symptoms of the *C. capsici* infecting bean plants is usually water-soaked lesions which advance and turn dark brown or black. These small lesions may coalesce which eventually leads to severe disease damage (Azad et al., 2020).

Table 5. Means of the disease severity percentage after 120 hours of inoculation of crude filtrate with fungal enzyme on bean pods

| **TREATMENTS** | **Hours of Incubation** | | | | | |
|---|---|---|---|---|---|---|
| | **12h** | **24h** | **48h** | **72h** | **96h** | **120h** |
| T1-control | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ |
| T2-25% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $1.25^{c}$ | $8.75^{bc}$ | $12.50^{b}$ |
| T3-50% CF | $0.00^{ns}$ | $0.00^{ns}$ | $2.50^{b}$ | $11.25^{b}$ | $13.75^{ab}$ | $13.75^{b}$ |
| T4-75% CF | $0.00^{ns}$ | $0.00^{ns}$ | $2.50^{b}$ | $8.75^{b}$ | $16.25^{ab}$ | $18.75^{ab}$ |
| T5-100% CF | $0.00^{ns}$ | $0.00^{ns}$ | $15.00^{a}$ | $20.00^{a}$ | $21.25^{a}$ | $26.25^{a}$ |
| Pr (> F) | - | - | 0.0046 | 0.0000 | 0.0025 | 0.0005 |

Values are the means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ means not significant

Study of Armesto et al., (2020), revealed that fungal plant pathogens are capable of secreting enzymes, enabling them to infect its host tissue. In their study, *Colletotrichum gloeosporioides* related to anthracnose disease were evaluated in producing hydrolytic enzymes. Their study resulted that all the enzymes mentioned were detected from the pathogen, and also obtained highest disease severity indexes, which suggested a relationship between enzymes and its aggressiveness of the isolates. Therefore, the present study further proved the activity of enzymes in disease development.

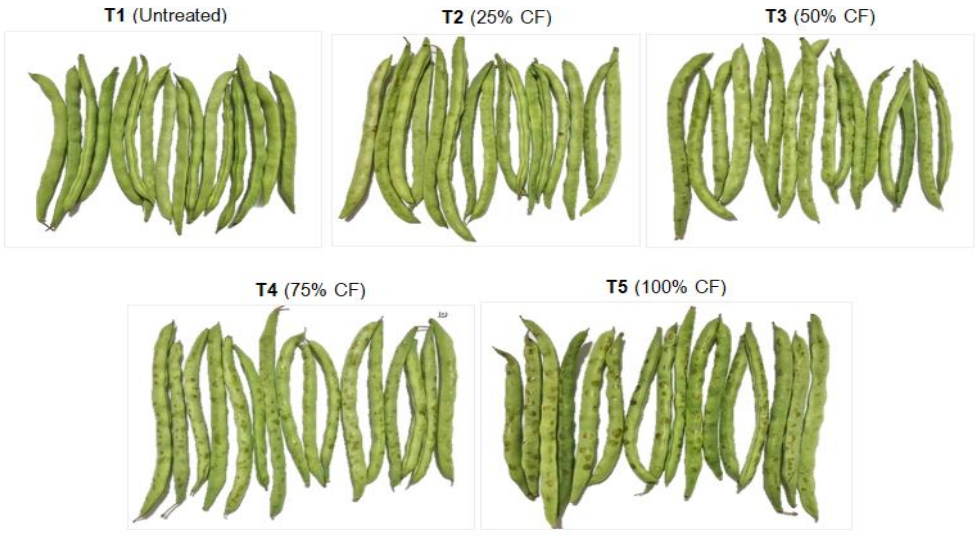



Figure 1. Bean pods (T1-T5) inoculated with different concentration of *Colletotrichum capsici* crude filtrate after 120 hours of inoculation

Meanwhile, for bell peppers (Figure 2), it revealed no infection on the first 48 hours (Table 6). On 72 hours, 75% CF, and 100% CF exhibited 6.67% and 11.67%, respectively. 50% CF exhibited 1.67% disease severity

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

while 75% CF and 100% CF remained. On 120 hours, 75% CF and 100% CF progressed with 13.33% and 21.67%, respectively while 50% CF remained at 1.67%. 25% CF remained no infection at all, and that the statistical analysis revealed that 100% CF differed significantly among all treatments except for 75% CF.

Table 6. Means of the disease severity percentage after 120 hours of inoculation of crude filtrate with fungal enzyme on bell pepper

| **TREATMENTS** | **Hours of Incubation** | | | | | |
|---|---|---|---|---|---|---|
| | **12h** | **24h** | **48h** | **72h** | **96h** | **120h** |
| T1-control | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ |
| T2-25% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $0.00^{b}$ | $0.00^{b}$ |
| T3-50% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{b}$ | $1.67^{b}$ | $1.67^{b}$ |
| T4-75% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $6.67^{ab}$ | $6.67^{ab}$ | $13.33^{ab}$ |
| T5-100% CF | $0.00^{ns}$ | $0.00^{ns}$ | $0.00^{ns}$ | $11.67^{a}$ | $11.67^{a}$ | $21.67^{a}$ |
| Pr (> F) | - | - | - | 0.0153 | 0.0282 | 0.0413 |

Values are means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ means not significant

Bell peppers has cuticle as barrier against fungal pathogen's infection in which the fruit tissues are rich in pectin and other polysaccharides, however, it can be degraded by the pathogen's enzymes. *C. capsici* infects bell peppers through penetration in the fruit's surface which is usually the stomata as the natural opening or through wounds as an artificial portal of entry (Krasnow and Ziv, 2022). Enzymes produced by the fungal pathogen particularly the pectinases and proteases break down the cell walls of its host, thereby disrupting the cellular integrity aiding to further invasion and colonization of the pathogen within the bell pepper fruit. Bell pepper infected with *C. capsici* exhibit sunken, and white to dark lesion which may be covered with fungal spores contributing to disease spread (Sangeetha et al., 2021).

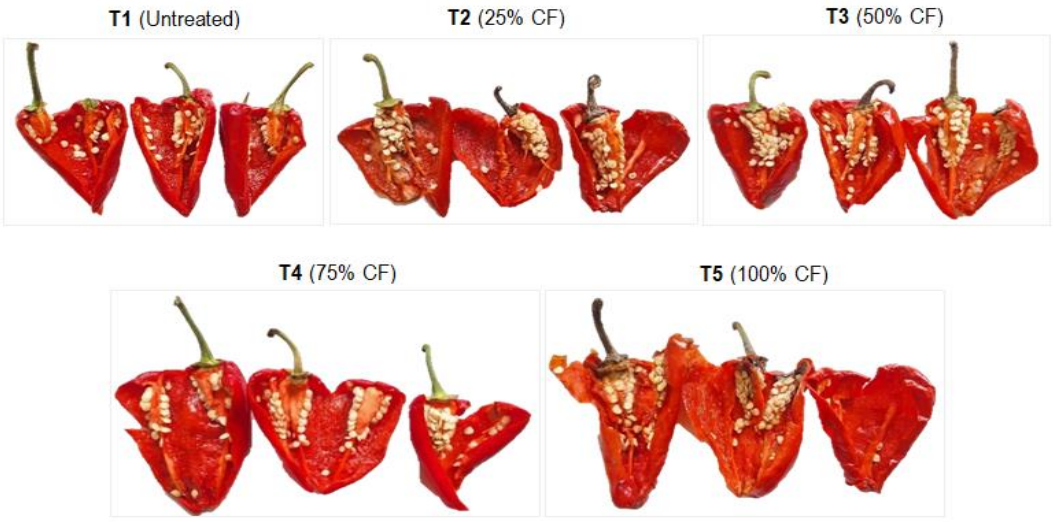



Figure 2. Bell pepper fruits (lengthwise cut) from T1-T5 inoculated with different concentration of *Colletotrichum capsici* crude filtrate after 120 hours of inoculation

On the other hand, similar trend was observed in soybean (Figure 3) where 50%, 75%, and 100% CF exhibited disease severity at 12 hours and progressed until 120 hours (Table 7). 25% CF exhibited disease severity on 48

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

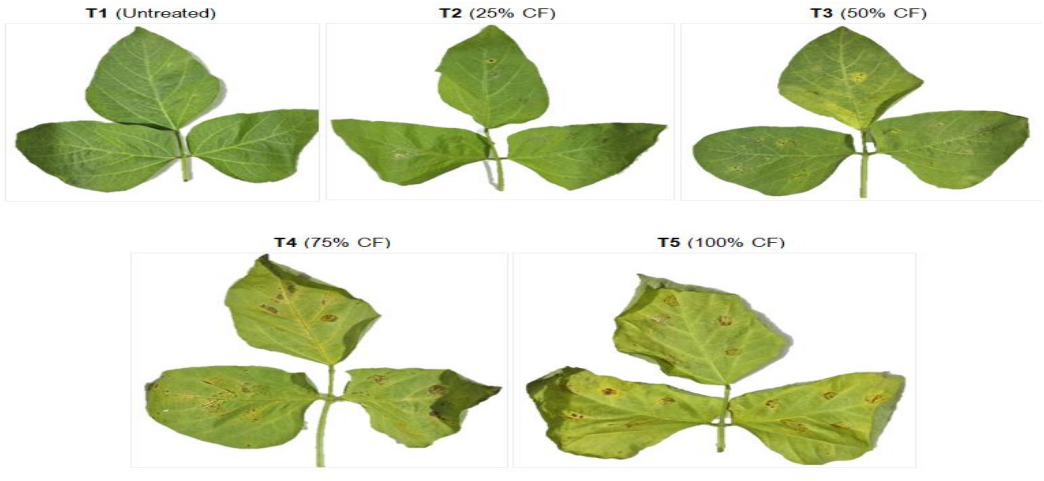
hours and progressed until 120 hours. After 120 hours, 100% CF showed the highest disease severity with 68.33%, followed by 50% CF with 51.57%, while 25% CF showed the lowest with 26.67%. Statistically, the results revealed that all treatments are comparable to each other with 100% CF as the highest implying that higher crude filtrate concentration with fungal enzymes aid in successful infection. Thus, understanding the interaction of the host plant and the enzymes secreted by the pathogen would contribute to disease management.

Table 7. Means of the disease severity percentage after 120 hours of inoculation of crude filtrate with fungal enzyme on soybean leaves

| **TREATMENTS** | **Hours of Incubation** | | | | | |
|---|---|---|---|---|---|---|
| | **12h** | **24h** | **48h** | **72h** | **96h** | **120h** |
| T1-control | $0.00^{b}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{c}$ | $0.00^{d}$ | $0.00^{d}$ |
| T2-25% CF | $0.00^{b}$ | $0.00^{c}$ | $11.67^{c}$ | $25.00^{b}$ | $26.67^{c}$ | $26.67^{c}$ |
| T3-50% CF | $1.67^{b}$ | $11.67^{b}$ | $25.00^{b}$ | $40.00^{b}$ | $43.33^{b}$ | $51.67^{b}$ |
| T4-75% CF | $15.00^{a}$ | $16.67^{b}$ | $33.33^{ab}$ | $40.00^{b}$ | $46.67^{b}$ | $48.33^{b}$ |
| T5-100% CF | $20.00^{a}$ | $30.00^{a}$ | $40.00^{a}$ | $56.67^{a}$ | $61.67^{a}$ | $68.33^{a}$ |
| Pr (> F) | 0.0000 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

Values are means of each treatment with four replications; means of the same superscript are not significantly different; and $^{ns}$ means not significant

Soybeans have a robust cuticle and its cell wall is composed with pectin, cellulose, and hemicelluloses which can be degrade during the infection process of *C. capsici* through producing enzymes, however as defense mechanism of the plant, soybean produces phytoalexins and other chemical compounds that could inhibit the growth of the pathogen as part of their defense to survive. Symptoms of anthracnose caused *by C. capsici* in soybean can be characterized as dark, and sunken lesions in which if it became severe, yield loss will be inevitable (Saxena et al., 2016). Study of Naveen et al., (2021), demonstrated that the fungal enzymes of *Colletotrichum* spp. such as the cellulase, pectin methylesterase, and ascorbate peroxidase was involved to the virulence of the pathogen. Their study resulted that all the mentioned enzymes were correlated with their pathogenicity which causes severe losses to the treated crops under favorable conditions, concluding the specific use of those enzymes as an indication of virulence of the pathogen.



ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

Figure 3. Soybean leaves (trifoliate) from T1-T5 inoculated with different concentration of *Colletotrichum capsici* crude filtrate after 72 hours of inoculation

The symptom characteristic developed on the crops used in this study can be further explained by the production of macerating enzymes of the pathogen which enabling them to successfully infect the host plants. For example, according to Anand et al., (2008), *Colletotrichum* spp. produces enzyme cellulases that catalyzes the host cell wall degradation. More so, according to Joshi, (2018), the fungal pathogen can produce pectinolytic enzyme, endo-polygalacturonases, protein kinases, glucanases, and chitinase. Furthermore, according to Villafana and Rampersad, (2020), the ability of *Colletotrichum* spp. to produce and secrete cutinase required to dismantle the cuticle of the host plant during penetration which is very crucial to the necrotrophic stage of their infection strategy. Cell wall-degrading enzymes (CWDEs) are critical for the initial infection processes as they degrade the complex carbohydrates components in the host plants' cell wall (Liao et al., 2012). And as the infection process is advancing, continuous degradation of pectin by the pectinases allows the pathogen to move, thereby spreading the infection such as the study of Prusky et al., (2007) which showed that the pectinases production of the microorganism is correlated with the extent of tissue colonization leading to symptom development of infected plants.

## CONCLUSION

This study demonstrates that crude filtrates (CF) of *Colletotrichum capsici* accelerate symptom development, increase disease incidence, and intensify severity in bean pods, bell peppers, and soybean leaves in a concentration-dependent manner. Higher CF levels consistently reduced the time to symptom appearance and led to greater infection levels, with soybean leaves showing the highest susceptibility compared to bean pods and bell peppers. These findings underscore the pivotal role of fungal enzymes in driving host tissue degradation and pathogenicity. The differential responses among hosts highlight the importance of host traits, such as stomatal density and defense gene expression, in influencing disease outcomes. Overall, the results provide new insights into enzyme-mediated infection processes of *C. capsici* and point to potential applications in resistance breeding and enzyme-targeted disease management strategies.

## RECOMMENDATION

The findings of this study can inform Integrated Pest Management (IPM) strategies by reducing reliance on synthetic fungicides and mitigating their environmental impact. Practical applications include modifying irrigation practices to lower humidity that favors fungal growth and adopting crop rotation to disrupt the life cycle of *C. capsici*. Given the global relevance of *Colletotrichum* spp. as pathogens of economically important crops, understanding their host interactions under changing climate conditions is essential. Future research should examine how temperature influences enzymatic activity and disease dynamics, with implications for food security, particularly in developing countries.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the University of Southeastern Philippines (USeP), Tagum-Mabini Campus, and the DA-Caraga Region RCPC for providing the resources and facilities necessary to conduct this research. Sincere appreciation is also extended to the authors' families and friends for their constant encouragement, understanding, and support throughout the duration of this work.

### Conflict of Interest

The authors declare that they have no conflict of interest in the publication of this research paper. No financial or personal relationships with other people or organizations have influenced the conduct of this research or the preparation of this manuscript.

### Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

request, as the dataset is not publicly archived.

## REFERENCES


1. Ali, M., Li, Q. H., Zou, T., Wei, A. M., Gombojav, G., Lu, G., & Gong, Z. H. (2020). Chitinase gene positively regulates hypersensitive and defense responses of pepper to Colletotrichum acutatum infection. *International Journal of Molecular Sciences*, *21*(18), 6624, https://doi.org/10.3390/ijms21186624
2. Armesto, C., Maia, F. G. M., Monteiro, F. P., & Abreu, M. S. D. (2020). Exoenzymes as a pathogenicity factor for Colletotrichum gloeosporioides associated with coffee plants. *Summa Phytopathologica*, *45*, 368-373, https://doi.org/10.1590/0100-5405/191071
3. Ayaa, D. B. (2021). Infection of Phaseolus vulgaris with the plant pathogen Colletotrichum species: A thesis submitted in partial fulfillment of the requirements for the Degree of Master at Lincoln University (Doctoral dissertation, Lincoln University), https://hdl.handle.net/10182/15005
4. Azad, C. S., Rautela, P., Gupta, S., and Singh, R. P. (2020). Major Diseases of Chili and Their Management. In *Diseases of Fruits and Vegetable Crops* (pp. 353-377). Apple Academic Press, eBook ISBN9780429322181, https://www.taylorfrancis.com/chapters/edit/10.1201/9780429322181-20/
5. Balendres, M. A. O. (2023). Plant diseases caused by fungi in the Philippines. Mycology in the Tropics, 163-188, https://doi.org/10.1016/B978-0-323-99489-7.00014-7
6. Ciha, A. J., & Brun, W. A. (1975). Stomatal size and frequency in soybeans 1. *Crop Science*, *15*(3), 309-313, https://doi.org/10.2135/cropsci1975.0011183X001500030008x
7. Dowling, M., Peres, N., Villani, S., & Schnabel, G. (2020). Managing Colletotrichum on fruit crops: A "complex" challenge. Plant Disease, 104(9), 2301-2316, https://doi.org/10.1094/PDIS-11-19-2378-FE
8. Freeman, S., Katan, T., & Shabi, E. (1998). Characterization of Colletotrichum species responsible for anthracnose diseases of various fruits. Plant disease, 82(6), 596-605, https://doi.org/10.1094/PDIS.1998.82.6.596
9. Gu, X., Cao, Z., Li, Z., Yu, H., & Liu, W. (2024). Plant immunity suppression by an β-1, 3-glucanase of the maize anthracnose pathogen *Colletotrichum graminicola*. BMC Plant Biology, 24(1), 339, https://doi.org/10.1186/s12870-024-05053-0
10. Huang, J. S. (2013). *Plant pathogenesis and resistance: Biochemistry and physiology of plant-microbe interactions*. Springer Science & Business Media, https://books.google.com.ph/books?hl=en&lr=&id=9BfvCAAAQBAJ&oi
11. Jeyaraj, A., Elango, T., Chen, X., Zhuang, J., Wang, Y., & Li, X. (2023). Advances in understanding the mechanism of resistance to anthracnose and induced defense response in tea plants. Molecular Plant Pathology, 24(10), 1330-1346, https://doi.org/10.1111/mpp.13354
12. Joshi, R. (2018). A Review on Colletotrichum spp. Virulence mechanism against host plant defensive factors. *J. Med. Plants Stud*, *6*(6), 64-67, DOI: https://doi.org/10.22271/plants.2018.v6.i6b.02
13. Kachroo, H. (2022). Endophytes: An Asset for Extracellular Hydrolases Production. In Endophyte Biology (pp. 149-183). Apple Academic Press, https://www.taylorfrancis.com/chapters/edit/10.1201/9781003277262-9
14. Krasnow, C., and Ziv, C. (2022). Non-chemical approaches to control postharvest gray mold disease in bell peppers. *Agronomy*, *12*(1), 216, https://doi.org/10.3390/agronomy12010216
15. Kubicek, C. P., Starr, T. L., & Glass, N. L. (2014). Plant cell wall–degrading enzymes and their secretion in plant-pathogenic fungi. *Annual review of phytopathology*, *52*, 427-451, https://doi.org/10.1146/annurev-phyto-102313-045831
16. Lakshmi, B. K. M., Reddy, P. N., & Prasad, R. D. (2011). Cross-infection potential of Colletotrichum gloeosporioides Penz. isolates causing anthracnose in subtropical fruit crops, http://www.pgia.pdn.ac.lk/files/Annual_congress/journel/V22/Paper/7.pdf
17. Liao, C. Y., Chen, M. Y., Chen, Y. K., Wang, T. C., Sheu, Z. M., Kuo, K. C., and Lee, M. H. (2012). Characterization of three Colletotrichum acutatum isolates from Capsicum spp. *European Journal of Plant Pathology*, *133*, 599-608, https://doi.org/10.1007/s10658-011-9935-7
18. Mehbub, I. (2006). Studies on the resistance of Solanum Melongena L against Colletotrichum Gloeosporioides (Penzig) Saccardo and control strategies of the Disease (Doctoral dissertation,

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue IX September 2025

University of North Bengal), https://ir.nbu.ac.in/server/api/core/bitstreams/b288f2c1-0a21-48bc-8f6b-7f1c520b92c1/content

19. Miller-Butler, M. A., Smith, B. J., Curry, K. J., & Blythe, E. K. (2019). Evaluation of detached strawberry leaves for anthracnose disease severity using image analysis and visual ratings. HortScience, 54(12), 2111-2117, https://doi.org/10.21273/HORTSCI14321-19
20. Naveen, J., Navya, H. M., Hithamani, G., Hariprasad, P., & Niranjana, S. R. (2021). Pathological, biochemical and molecular variability of Colletotrichum truncatum incitant of anthracnose disease in chilli (Capsicum annuum L.). *Microbial pathogenesis*, *152*, 104611, https://doi.org/10.1016/j.micpath.2020.104611
21. Prajapati, V. S., Trivedi, U. B., & Patel, K. C. (2014). Kinetic and thermodynamic characterization of glucoamylase from Colletotrichum sp. KCP1. *Indian journal of microbiology*, *54*, 87-93, https://doi.org/10.1007/s12088-013-0413-0
22. Pring, R. J., Nash, C., Zakaria, M., & Bailey, J. A. (1995). Infection process and host range of Colletotrichum capsici. *Physiological and Molecular Plant Pathology*, *46*(2), 137-152, https://doi.org/10.1006/pmpp.1995.1011
23. Prusky, D., Gold, S., & Keen, N. T. (1989). Purification and characterization of an endopolygalacturonase produced by Colletotrichum gloeosporioides. *Physiological and Molecular Plant Pathology*, *35*(2), 121-133, https://doi.org/10.1016/0885-5765(89)90082-9
24. Riseh, R. S., Vatankhah, M., Hassanisaadi, M., & Barka, E. A. (2024). Unveiling the Role of Hydrolytic Enzymes from Soil Biocontrol Bacteria in Sustainable Phytopathogen, https://doi.org/10.31083/j.fbl2903105
25. Sangeetha, C. G., Devappa, V., Snehalatharani, A., Navyashree, S. E., and Sundeep, T. (2021). Postharvest Diseases of Capsicum sp. and Brinjal and Their Management. In *Postharvest Handling and Diseases of Horticultural Produce* (pp. 337-348). CRC Press, https://www.taylorfrancis.com/chapters/edit/10.1201/9781003045502-29
26. Saxena, A., Raghuwanshi, R., Gupta, V. K., and Singh, H. B. (2016). Chilli anthracnose: the epidemiology and management. *Frontiers in microbiology*, *7*, 215999, https://doi.org/10.3389/fmicb.2016.01527
27. Silva, M. A. D., Santos, C., Pérez-Nevado, F., Lima, N., & Bentes, J. L. D. S. (2021). Enzymatic activity and process of initial infection of guarana plant (Paullinia Cupana) by pathogenic and endophytic strains of *Colletotrichum guaranicola*. *Revista fitotecnia mexicana*, *44*(1), 67-75, https://doi.org/10.35196/rfm.2021.1.67
28. Thaker, A. (2022). Functional Characterization of Genes Involved in Pathogenicity of the Rice Blast Fungus Magnaporthe oryzae (Doctoral dissertation, Maharaja Sayajirao University of Baroda (India), https://www.proquest.com/openview/729d475e2cf75bb352059a9e09623943
29. Villafana, R. T., & Rampersad, S. N. (2020). Diversity, structure, and synteny of the cutinase gene of Colletotrichum species. *Ecology and evolution*, *10*(3), 1425-1443, https://doi.org/10.1002/ece3.5998
30. Wijesundera R.L.C., J.A. Bailey, R.J.W. Byrde, A.H. Fielding (1989). Cell wall-degrading enzymes of *Colletotrichum lindemuthianum*: their role in the development of bean anthracnose, Physiological and Molecular Plant Pathology, Volume 34, Issue 5, Pages 403-413, ISSN 0885-5765, https://doi.org/10.1016/0885-5765(89)90067-2
31. Yokota, K., Teraoka, T., Tsujii, Y., Suzuki, H., Murakami, K., Miwa, E., & Higuchi, K. (2010). Effect of high molecular weight carbohydrates on bud cell formation by Fusarium oxysporum in potato dextrose broth. Journal of general plant pathology, 76, 219-224, https://doi.org/10.1007/s10327-010-0238-1
32. Zhang, S., Li, C., Si, J., Han, Z., & Chen, D. (2022). Action mechanisms of effectors in plant-pathogen interaction. International Journal of Molecular Sciences, 23(12), 6758, https://doi.org/10.3390/ijms23126758