



# Inhibitory Effect of the Extracts from Sunflower Disk and Stalk on *Fusarium Sulphureum* Causing Potato Dry Rot

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## Abstract

Potato (*Solanum tuberosum* L.) dry rot due to fungal infections causes the loss of a significant amount of potatoes. In this study, the antifungal effects of sunflower (*Helianthus annuus* L.) extracts against *Fusarium sulphureum* were investigated. The results of in vitro antifungal tests showed that all four sunflower extracts significantly inhibited the growth of *F. sulphureum*. Notably, the optimal inhibitory concentrations of ethyl acetate extract from sunflower disk (EESD), ethyl acetate extract from sunflower stalk (EESS), petroleum ether extract from sunflower disk (PESD) and petroleum ether extract from sunflower stalk (PESS) against *F. sulphureum* were 160 mg/mL, 160 mg/mL, 240 mg/mL, and 240 mg/mL, respectively. In addition, these extracts significantly inhibited the activities of polygalacturonase (PG), polymethylgalacturonase (PMG), carboxymethyl cellulase (Cx), and  $\beta$ -glucosidase ( $\beta$ -Glu) secreted by *F. sulphureum* in three potato varieties: Longshu No.7, Longshu No.10 and Xindaping. These results provide a theoretical foundation for the biological control of potato dry rot.

**Keywords** Sunflower disk · Sunflower stalk · *Fusarium sulphureum* · Potato dry rot · Cell wall degrading enzyme

## Abbreviations

CK	Blank control
CWDEs	Cell wall degrading enzymes
Cx	Carboxymethyl cellulase
EESD	Ethyl acetate extract from sunflower disk
EESS	Ethyl acetate extract from sunflower stalk
PDA	Potato dextrose agar
PESD	Petroleum ether extract from sunflower disk
PESS	Petroleum ether extract from sunflower stalk
PG	Polygalacturonase
PMG	Polymethyl-galacturonase
$\beta$ -Glu	$\beta$ -glucosidase

## Introduction

Potato (*Solanum tuberosum* L.) has been viewed as an important food crop worldwide (Amin et al. 2023; Kumar et al. 2021). However, potato dry rot, which is caused by over 13 species of *Fusarium* has posed a huge threat to potato production (Du et al. 2012; Gachango et al. 2012; Heltoft et al. 2016; Pan et al. 2023; Recep et al. 2009). *Fusarium* spp. is a species of significant phytopathogenic fungi that can easily infect potato tubers via wounds during harvesting, storage, and transportation (Bao et al. 2014; Liu et al. 2022). Besides, *Fusarium sulphureum* is a commonly occurring fungal pathogen in America, Europe, and China, posing a significant risk to potato crops in these regions (Li et al., 2014; Li et al. 2023).

*F. sulphureum* can invade the tissue of potato tubers through lenticels, bud eyes, and wounds (Yang et al. 2022). During the invasion of the host, *F. sulphureum* secretes a suite of cell wall degrading enzymes (CWDEs) that aid in the degradation of the plant cell wall structure, facilitating colonization within the host cells and subsequent infection (Wilfried et al. 2009). Notably, in potato tubers infected with *F. sulphureum*, polygalacturonase (PG), polymethylgalacturonase (PMG), carboxymethyl cellulase (Cx), and

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$\beta$ -glucosidase ( $\beta$ -Glu) exhibit significant activities, surpassing the influence of polygalacturonic acid trans-eliminase, pectin methyl-trans-eliminase, pectin methylesterase, and pectate lyase in the infected tissues (Yang et al. 2012). In addition, *F. sulphureum* can also produce a variety of mycotoxins, mainly trichothecenes, which can inhibit cell aerobic respiration and destroy cell membranes, further destroying host cells and absorbing nutrients, resulting in rotting and drying up of potato tubers (Fan et al. 2021; Xue et al. 2014).

So far, the control of potato dry rot has primarily relied on the application of chemically synthesized fungicides. (Sandipan et al. 2016). Unfortunately, due to various issues such as chemical fungicide residues, the emergence of fungicide resistance among pathogens, potentially harmful effects on human and animal health, and environmental pollution, researchers have been exploring innovative and sustainable approaches to prevent plant diseases (Xue et al. 2018, 2019).

Botanical fungicides are abundant, natural, biodegradable, and renewable resources with antimicrobial activity against *F. sulphureum* (Li et al. 2023). Currently, researchers have examined the antifungal activity of onion peel extract and potato glycoalkaloids derived from potato peel against *F. sulphureum*, with the highest inhibitory capacity observed at concentrations of 5 g/mL and 200 mL/L, respectively, surpassing those observed at lower concentrations (Li et al. 2023; Qiu et al. 2017). Li et al. (2014) also demonstrated the control properties of the essential oil of *Zanthoxylum bungeanum* on *F. sulphureum*, with a minimum inhibitory concentration of 6.25%. Although some progress has been made in the research on the biological control of potato dry rot, there is still a lack of an effective biological fungicide to prevent and control the disease (Liu et al. 2020).

Sunflower (*Helianthus annuus* L.) is one of the three primary oil crops in the world (Grazielle et al., 2020; Wu et al. 2022). However, the main by-products of the sunflower industry, such as sunflower stalks and disks, are discarded, resulting in resource waste (Daraee et al. 2018; Yang et al. 2020; Zhao et al. 2023). Sunflower contains sesquiterpenes, diterpenes, triterpenes, lignans, flavonoids, phenylpropanoid, steroids, and other chemical components, with biological activities like antibacterial, anti-tumor, and antioxidant (Alica et al. 2021; Li et al. 2020; Muhammad et al. 2018). Currently, sunflower extracts have been demonstrated to possess inhibitory properties against various common pathogenic bacteria and fungi, including *Staphylococcus aureus*, *Escherichia coli*, and *Alternaria alternata* (Ilori et al. 2022; Li et al. 2019).

In this study, two polar solvents, ethyl acetate and petroleum ether, were used to extract secondary metabolites from sunflower disks and sunflower stalks, respectively. So far, there has been no report on the effects of sunflower extract on

*F. sulphureum*. Therefore, this research aimed to explore the antifungal potential of sunflower extracts against important fungi causing potato dry rot in in vitro conditions. In addition, to further validate the antifungal activity of sunflower extracts on *F. sulphureum*, we evaluated their impact on the activities of PG, PMG, Cx, and  $\beta$ -Glu in infected potato tuber slices. To comprehensively elucidate the significance of sunflower extract applications across diverse potato cultivars, the present study randomly selected three potato varieties (Longshu No.7, Longshu No.10, and Xindaping) native to Gansu Province for experimental investigation.

## Materials and Methods

### Materials and Pathogen

Dried sunflower disks and sunflower stalks were provided by Jingye Sunflower Industrial Park Co., Ltd. (Lanzhou, Gansu, China). Longshu No.7, Longshu No.10, and Xindaping were obtained from the Potato Research Center of Gansu Academy of Agricultural Sciences (Lanzhou, Gansu, China).

*F. sulphureum* was donated by Gansu Agricultural University (Lanzhou, Gansu, China). The pathogen was inoculated on potato dextrose agar medium (PDA) at 28 °C before use.

### Preparation of Sunflower Extracts

The dried sunflower disks and stalks were crushed and sieved through a 65-mesh sieve. They were then respectively mixed with ethyl acetate and petroleum ether, at a ratio of material to liquid was 1:10. The mixtures were allowed to stand for 5 days, followed by centrifugation at 4,000 rpm for 15 min. The supernatants were concentrated in a rotary evaporator (RE5203, Shanghai Yarong Biochemical Instrument Factory) and then freeze-dried to obtain the original solutions of sunflower extracts.

### Effects of Sunflower Extracts on the Diameter of the *F. Sulphureum* Colony

The extract solutions from sunflower disks and stalks with concentrations of 10, 20, 40, 80, 160, 240, and 320 mg/mL were prepared using ethyl acetate and petroleum ether respectively. Each of the four extracts—ethyl acetate extract from sunflower disk (EESD), ethyl acetate extract from sunflower stalk (EESS), petroleum ether extract from sunflower disk (PESD) and petroleum ether extract from sunflower stalk (PESS)—was coated evenly on PDA at separate concentrations of 0, 10, 20, 40, 80, 160, 240, and 320 mg/mL,

using 1 mL of the respective extract solution for each concentration (Zhao et al. 2016). Three 6-mm-diameter blocks of *F. sulphureum* were put into each PDA plate, and then the plates were incubated at 28 °C for 24 h. The diameters of pathogen colonies were measured by the method of crossing with a vernier caliper and the average values were calculated (Li et al. 2019). Each had three replicates.

## Effects of Sunflower Extracts on CWDE Activities

### Treatment of Potato Tubers

The potatoes of Longshu No.7, Longshu No.10, and Xindaping were peeled. The potato tubers were then sterilized using 0.1% sodium hypochlorite for 20 min and rinsed in sterile water. Slices of potato tuber (50 mm in diameter and 1 cm in thickness) were made with a sterile knife. The slices were placed on sterile wet filter paper and incubated in the dark for 1 h.

The randomly distributed potato slices were subsequently coated evenly with 0.1 ml of EESD, EESS, PESD, and PESS. The concentrations of EESD and EESS were fixed at 0, 140, 160, and 180 mg, respectively, while the concentrations of PESD and PESS were adjusted to 0, 220, 240, and 260 mg/mL, respectively. Then, *F. sulphureum* grown on PDA for 7 days with a diameter of 6 mm was placed on potato slices with the hyphae side in contact. The slices with PDA blocks of identical size to the *F. sulphureum* blocks were regarded as the blank control (CK). Each treatment had three replicates.

### Preparation of CWDE Extracts

Three grams of potato tuber slices, which had been incubated at 28 °C for 2 days, were mixed with 6 mL of 95% ethanol. The homogenates were ground in an ice bath and then placed at 4 °C for 10 min. They were centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatants were poured out. The precipitates were mixed with 3 mL of pre-cooled 80% ethanol and then kept at 4 °C for 10 min. The mixtures were centrifuged again. After the supernatants were poured out once more, 5 mL of extraction buffer (acetic acid-sodium acetate buffer solution with a concentration of 50 mmol/L and pH of 5.5, containing 1.8 mol/L NaCl) was added to the precipitates. After centrifugation, the CWDE extracts were obtained and stored at 4 °C before use. Each treatment had three replicates.

### Determination of the Activities of PG and PMG

The determination of the activities of PG and PMG was carried out according to Cao et al. (2007) with some

modifications. In brief, 0.5 mL of CWDE extract was combined with 1.0 mL of acetic acid-sodium acetate buffer with a concentration of 50 mmol/L and pH of 5.5 and 0.5 mL of 10 g/L substrate (substrate of PG: polygalacturonic acid; substrate of PMG: pectin). The mixture was incubated at 37 °C for 1 h. Subsequently, 1.5 mL of 3,5-dinitrosalicylic acid was added, the mixture was boiled for 5 min, and then rapidly cooled to room temperature. The absorbance was measured at a wavelength of 540 nm. The standard curve was constructed based on the mass and absorbance of glucose. The activities of PG and PMG were calculated based on the amount of reducing sugar released by enzymatic hydrolysis. The enzyme activities of PG and PMG were calculated by the following formula:

$$\text{enzyme activity (mg/h} \cdot \text{g)} = \frac{1.08m'V}{V_s t m}$$

where  $m'$  is the mass of glucose from the standard curve (mg),  $V$  is the total volume of sample extraction solution (mL),  $V_s$  is the volume of sample extraction solution used for the determination (mL),  $t$  is the enzymolysis time (h),  $m$  is the sample mass (g), and 1.08 is the coefficient of glucose converted to galacturonic acid (194/180).

### Determination of the Activities of Cx and $\beta$ -Glu

1.5 mL of 10 g/L substrate (substrate of Cx: sodium carboxymethyl cellulose; substrate of  $\beta$ -Glu: salicin) was chosen and processed according to the method outlined for the determination of PG and PMG activities. The enzyme activities of Cx and  $\beta$ -Glu were calculated by the following formula:

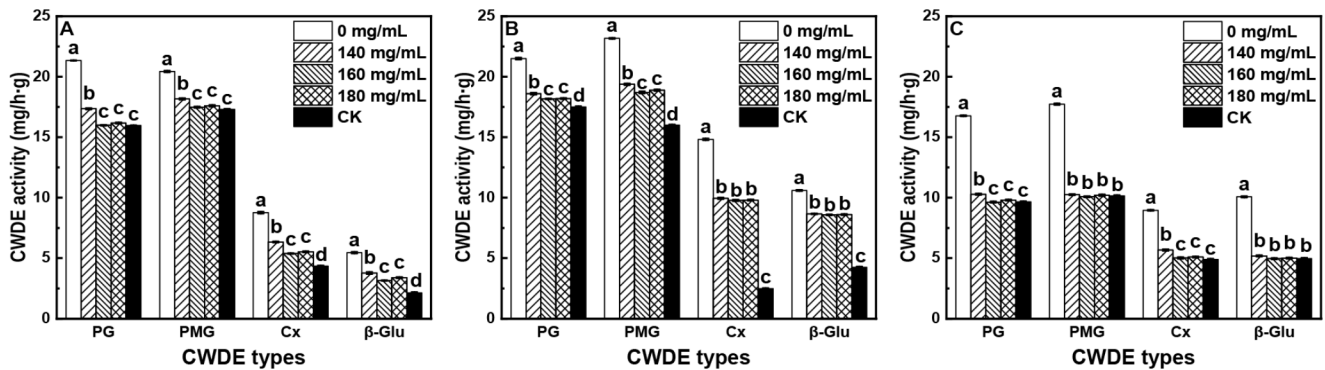
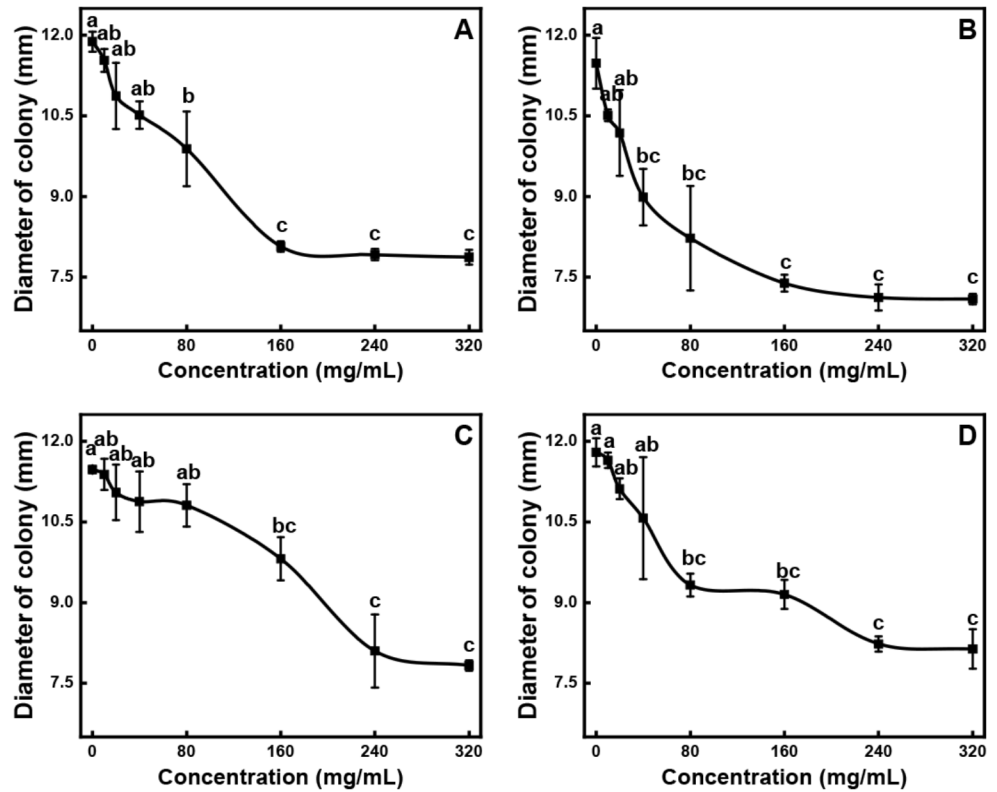
$$\text{enzyme activity (mg/h} \cdot \text{g)} = \frac{m'V}{V_s t m}$$

where  $m'$  is the mass of glucose from the standard curve (mg),  $V$  is the total volume of sample extraction solution (mL),  $V_s$  is the volume of sample extraction solution used for the determination (mL),  $t$  is the enzymolysis time (h), and  $m$  is the sample mass (g).

### Statistical Analysis

The experiment was repeated at least three times. Data were subjected to the analysis of variance (ANOVA) using IBM SPSS software. All values were presented as the mean  $\pm$  standard error of at least three independent experiments, and differences showing  $p < 0.05$  were considered significant. Figures were created using Origin 8.5 software.

**Fig. 1** The diameter of the *F. sulphureum* colony treated with (A) EESD, (B) EESS, (C) PESD, and (D) PESS. Different letters indicate significant differences in the diameter of the colony ( $p < 0.05$ )



**Fig. 2** Effects of EESD on the activities of CWDEs secreted by *F. sulphureum* infecting (A) Longshu No.7, (B) Longshu No.10, and (C) Xindaping. Different letters indicate significant differences in the activity of CWDE ( $p < 0.05$ )

## Results

### Effects of Sunflower Extracts on the Diameter of the *F. Sulphureum* Colony

In the concentration range of 10 to 160 mg/mL, the diameter of the *F. sulphureum* colony decreased significantly with the increase of EESD and EESS concentrations (Fig. 1A, B). However, there was no significant difference in the diameter of the colony at concentrations above 160 mg/mL.

In the concentration range of 10 to 240 mg/mL, the diameter of the fungal colony exhibited a negative correlation with the concentration of PESD and PESS (Fig. 1C, D).

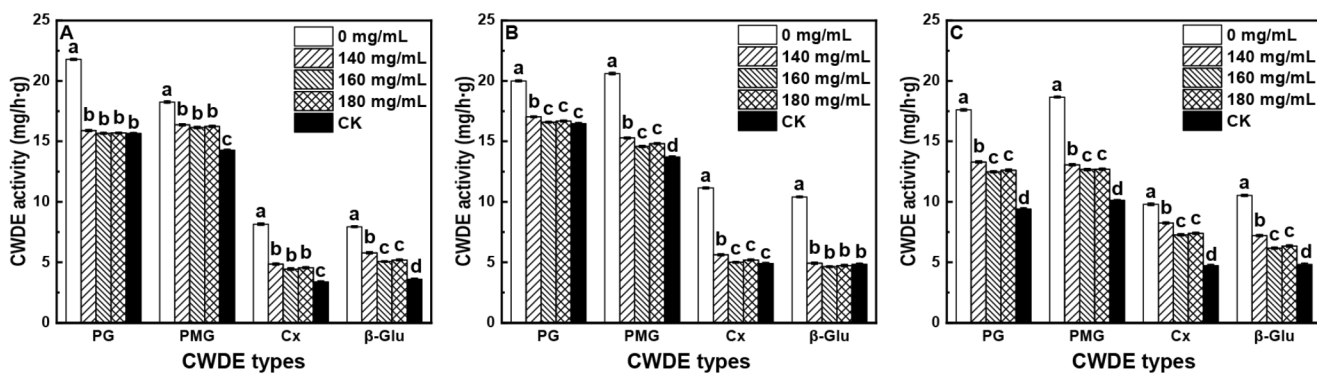
However, there was no significant change in the concentration range of 240 to 320 mg/mL.

### Inhibition of Sunflower Extracts on the Activities of CWDEs

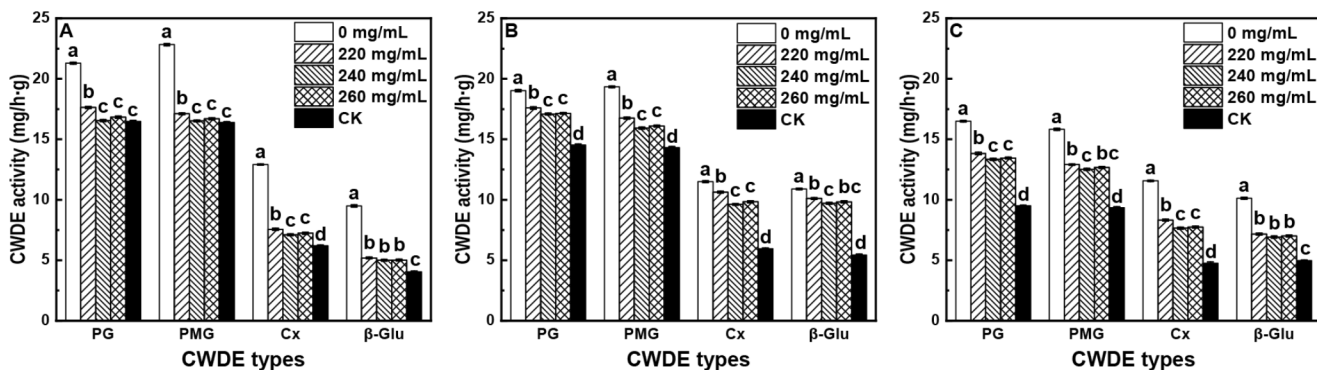
#### Inhibition of EESD on the Activities of CWDEs

As shown in Fig. 2, the application of EESD led to a notable reduction in the activities of PG, PMG, Cx, and  $\beta$ -Glu, produced by *F. sulphureum* infecting tubers of Longshu No.7, Longshu No.10, and Xindaping, when compared to the





**Fig. 3** Effects of EESS on the activities of CWDEs secreted by *F. sulphureum* infecting (A) Longshu No.7, (B) Longshu No.10, and (C) Xindaping. Different letters indicate significant differences in the activity of CWDE ( $p < 0.05$ )



**Fig. 4** Effects of PESD on the activities of CWDEs secreted by *F. sulphureum* infecting (A) Longshu No.7, (B) Longshu No.10, and (C) Xindaping. Different letters indicate significant differences in the activity of CWDE ( $p < 0.05$ )

control without EESD. The optimal inhibitory concentration of EESD was 160 mg/mL.

At concentrations of 160 and 180 mg/mL, the activities of PG and PMG treated with EESD were at the same level as those of CK, which meant that EESD could reduce the activities of PG and PMG secreted by *F. sulphureum* in Longshu No.7 to the normal level without infection (Fig. 2A). In the tissue of Xindaping, the activities of PG and Cx were at the same level as their respective CK at concentrations of EESD at 160 and 180 mg/mL (Fig. 2C). Furthermore, the activities of PMG and β-Glu in all EESD-treated groups were also at the same level as their respective CK. These results indicate that EESD effectively reduced the activities of the four CWDEs in the tuber of Xindaping to the normal level that are not significantly different from samples without pathogenic fungal infection.

**Inhibition of EESS on the Activities of CWDEs**

EESS could significantly inhibit the activities of four kinds of CWDEs secreted by *F. sulphureum* on Longshu No.7, Longshu No.10, and Xindaping, with the optimal inhibitory concentration being 160 mg/mL (Fig. 3). Furthermore, EESS could reduce the activity of PG on Longshu No.7 and

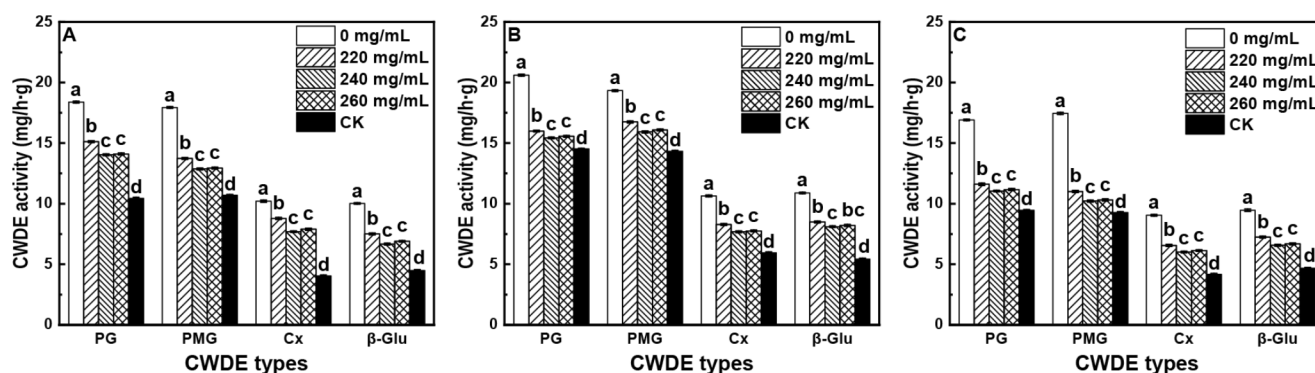
the activities of PG, Cx, and β-Glu on Longshu No.10 to levels not significantly different to samples without *F. sulphureum* infection (Fig. 3A, B).

**Inhibition of PESD on the Activities of CWDEs**

After PESD treatment, the activities of four kinds of CWDEs secreted by *F. sulphureum* on three kinds of potato were significantly reduced, with the optimal inhibitory concentration of PESD being 240 mg/mL (Fig. 4). Meanwhile, the addition of PESD reduced the activities of PG and PMG in Longshu No.7 to levels without infection of the pathogen (Fig. 4A).

**Inhibition of PESS on the Activities of CWDEs**

On the three potato varieties, the activities of four kinds of CWDEs secreted by *F. sulphureum* declined markedly following PESS treatment, and the optimal inhibitory concentration of PESS was 240 mg/mL (Fig. 5).



**Fig. 5** Effects of PESS on the activities of CWDEs secreted by *F. sulphureum* infecting (A) Longshu No.7, (B) Longshu No.10, and (C) Xindaping. Different letters indicate significant differences in the activity of CWDE ( $p < 0.05$ )

## Discussion

Figure 1 reveals a negative correlation between the diameter of the *F. sulphureum* colony and the concentrations of EESD, EESS, PESD, and PESS, indicating that the four sunflower extracts have a significant inhibitory effect on the growth of *F. sulphureum*. Combined with the factor of cost, the optimal inhibitory concentrations for EESD and EESS were 160 mg/mL, while those for PESD and PESS were 240 mg/mL. In our research, compared with the fraction of petroleum ether, the fraction of ethyl acetate of sunflower achieved the best effect at a lower concentration.

It can be seen from Figs. 2, 3, 4 and 5 that, the activities of pectinase (PG and PMG) on Longshu No.7, Longshu No.10, and Xindaping exceeded those of cellulase (Cx and  $\beta$ -Glu), which is consistent with the findings reported by Yang et al. (2012) on Longshu No.3.

According to the results, both non-inoculated and *F. sulphureum*-infected potato tissues produce CWDEs. Notably, the activities of CWDEs in potato tissues inoculated with *F. sulphureum* were significantly higher than those of CK. This finding aligns with previous research on corn stalk rot and root rot of *Panax ginseng*, indicating the presence of CWDEs in both healthy and diseased tissues, yet a notable surge in enzymatic activity is exclusively evident in the latter (Feng et al. 2022; Gao et al. 2000).

Notably, the application of all four sunflower extracts resulted in a decrease in the activities of the four CWDEs on three types of potato inoculated with *F. sulphureum*, indicating that the sunflower extracts can mitigate the impacts of *F. sulphureum* infection on potato tissues. It appears that the decrease in enzyme activity at inhibitory concentrations of sunflower extracts is primarily due to the reduction in pathogenic fungi growth caused by these extracts.

Furthermore, our research also unveiled significant variations in the activities of PG, PMG, CX, and  $\beta$ -Glu among different potato cultivars, both in their uninoculated (healthy) tissues and those inoculated with *F. sulphureum*.

This variation in baseline enzyme activities may indicate inherent differences in disease susceptibility or resistance mechanisms among the potato cultivars. The application of sunflower extracts at varying concentrations further modulated the activities of these CWDEs to varying degrees. These discrepancies in enzyme activities could potentially stem from multiple factors, including genetic variations among potato cultivars, diverse environmental conditions during cultivation, varying developmental stages, unique stress responses, and possibly other regulatory mechanisms that are yet to be identified. Consequently, further research is imperative to elucidate the specific mechanisms underlying these differences and assess their potential implications for disease development and management in potato crops.

In conclusion, this study may have implications for the prevention and control of potato dry rot and the comprehensive utilization of sunflower by-products. However, this experiment serves merely as a preliminary exploration into the potential of sunflower by-products in the realm of potato dry rot control. Further validation throughout the growth process of potato plants is warranted to substantiate the findings.

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## Declarations

**Conflict of Interest** The authors have no conflicts of interest to declare. All experiments within this study comply with current laws within China in which they were conducted.

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