# Allelopathy of Wheat and Faba Bean Extracts in an Intercropping System

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## Abstract

# Background

We intensively studied faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) intercropping and found that this type of intercropping can effectively control the occurrence of faba bean wilt under field conditions. We conducted hydroponic experiments to explore the role of plant extracts in process of soil-borne diseases and the mechanism of disease control of faba bean and wheat intercropping. In this experiment, three concentration gradients of faba bean and wheat stems and leaves and root extracts were added to study the effects of faba bean and wheat extracts on faba bean growth, root physiological resistance and the growth of *Fusarium oxysporum* f. sp. *fabae* (FOF).

# Result

Faba bean extracts significantly inhibited the growth of faba bean seedlings and the activity of root defense enzymes and significantly stimulated the growth of FOF at high concentrations. Compared with the treatment with faba bean extracts, wheat extracts significantly enhanced the growth of faba bean seedlings, increased the activity of defense enzymes and inhibited the growth of FOF.

# Conclusions

Based on these results, we believe that wheat extract can effectively alleviate the autotoxicity of faba beans and also control the occurrence of faba bean wilt in the field. This provides a theoretical basis for practical intercropping to reduce the harm of faba bean wilt.


## Background

Continuous planting and harvest of single crops is a common practice in modern agriculture, and that has resulted in serious obstacles. The hazards of continuous cropping obstacles primarily include soil compaction, the frequent occurrence of soil-borne diseases, a reduction in crop yields or even a total lack of germination of the seeds. Among them, the frequent occurrence of soil-borne diseases has always been a very difficult problem during actual production (Young, C.C., 1984; Grodzinsky, A.M., 1992). Thus far, soil-borne diseases have seriously threatened the production of various cash crops, such as watermelon, peanut, and cotton, which has a substantial impact on agricultural production around the world (Xiao-Gang, L et al,2013;Li, X. G2014Li, X et al,2017). The accumulation of autotoxic substances has always been a central area of research in the study of the causes of frequent occurrence of soil-borne diseases. Many studies have shown that the accumulation of autotoxic substances strongly promotes the occurrence of soil-borne diseases (Asaduzzaman, M.et al, 2012). For instance, the secretion of phenolic acids, such as cinnamic acid, coumaric acid and ferulic acid, from cucumber roots, and the products of decomposition of cucumber increase the risk of Fusarium wilt (Hao, Z. P et al, 2006; Ohno, S

et al, 2001; Yu, J. Q et al, 2003). The accumulation of autotoxic substances in the rhizosphere during peanut monocropping aggravates the occurrence of soil-borne diseases of peanut (Xiao-Gang, L et al 2013). Other studies have shown that the main reason that autotoxic substances can promote the occurrence of diseases is that they can have a strong destructive effect on plant physiological and biochemical resistance. For example, Ye SF et al found that cinnamic acid in cucumber autotoxic substances destroyed the plant antioxidant system, increased the content of active oxygen free radicals in the root and accelerated the degree of membrane lipid peroxidation, thereby rendering the plants more susceptible to infection and increasing the incidence of disease. Several chemical and biological methods have been developed to control plant diseases (Gil VS et.al, 2008). However, these methods are not environmentally friendly or sufficiently efficient (Minuto A et al, 2006; Li, X.et al, 2018).

Intercropping is a planting method that planting two or more crops in close proximity (Li, X.et al, 2018). In actual production, it is used as a green and efficient planting method to control soil-borne diseases and increase the yields of crops (Ren, L et al,2016 Li, X. G,2014). Allelopathy is an indispensable part of the study of the disease control mechanism of intercropping. For example, in the wheat/watermelon intercropping system, wheat allelopathic substances secreted by the root system increase the expression of watermelon defense genes, improve the ability of watermelon to resist the invasion of pathogen and control the occurrence of wilt (Huifang, L et al 2018). In the intercropping system of cumin (*Cuminum cyminum* L.) and watermelon, cumin acid secreted by the root system of *C. cyminum* significantly increased the activity of antioxidant enzymes and defensive enzymes in the watermelon roots and improved the ability of watermelon to resist pathogen (Sun, Y et al,2017). Allelopathic chemicals can enter the environment in different manners to play a role in the direct or indirect effects on growth of plants. The primary manners in which allelopathic chemicals are released include following their release by aboveground volatilization and leaching, secretion by roots and the decay of stubble (Guo, K et al 2016). Root secretion is the main source of allelochemicals belowground; these substances enter the soil directly through secretions from the plant roots and play a role (Ren, L et al,2016; Asaduzzaman, M. et al, 2012; Xiao-Gang, L et al 2013). The study of other allelopathic substances in plants is usually conducted with plant extracts. Many studies have found that the extracts of rock rose (*Cistus ladanifer*), wheat (*Triticum aestivum* L.), sunflower ( *Helianthus annuus Linn.* ) and alfalfa (*Medicago sativa Linn.*) plants have a strong allelopathic effect on their own physiology or that of other plants (J. C. Alías et al ,2006 Hanwen Wu et al,2007 Tesio, F et al,2012 Eldarier et al,2011). However, most of the research on the mechanism of disease control by intercropping focuses on plant root exudates, and there are few studies on the extracts of plant stems, leaves and roots that also have allelopathic effects.

Faba beans are widely cultivated worldwide as an important legume crop (Alghamdi S S.et al, 2012). However, because of the continuous single planting, the yield of faba beans is greatly reduced owing to Fusarium wilt (Stoddard et al, 2010). In Yunnan and southwestern China, faba beans are usually planted with wheat to control faba bean wilt. We intensively studied the mechanism of control of faba bean and wheat intercropping to control the wilt disease of faba bean. We explained the mechanism of control of faba bean and wheat intercropping from the aspect of biodiversity. However, there is a lack of data on the ability of plant extracts to cause disease. We conducted a preliminary experiment on the allelopathy of

extract of faba bean stems and leaves based on a field experiment but using hydroponics. In this study, we aimed to (i) reveal the allelopathic capability of extracts from faba beans and wheat; (ii) explore the mechanism of disease control from a wheat and faba bean intercropping system and lay the groundwork for further research; and (iii) provide effective theoretical guidance for actual agricultural production to achieve the most effective disease control.

# Results

# Effect of intercropping wheat and faba bean on Fusarium wilt of faba bean

Figure 2A shows that the incidence of faba bean wilt during the mature and flowering periods was significantly higher than that during the branching period in the monocropping and intercropping models. Compared with monocropping, intercropping wheat and faba bean significantly reduced the incidence of faba bean wilt in the branching and flowering stages by 20.63% and 13.36%., respectively

In Fig. 2B, the disease index of faba bean wilt during the flowering stage is significantly higher than that during the branching stage, and the disease index in mature stage of faba bean wilt is significantly higher than that in the flowering stage. The disease index gradually increases with time. Compared with monocropping, intercropping wheat and faba bean significantly reduced the disease index of faba bean wilt by 51.64%, 37.78% and 29.72% during the branching stage, fiowering stage and mature stage, respectively. Figure 2AB shows that intercropping with faba bean and wheat can effectively control the faba bean wilt compared with the faba bean monocropping, and the effect is particularly significant in the suppression of faba bean wilt disease index. The branching period is the period when the faba bean and wheat intercropping is the most effective at controlling disease. The incidence of faba bean wilt and the disease index decreased by 20.63% and 51.64% respectively.

### Effects of wheat and faba bean stem, leaf and root extracts on faba bean growth

As shown in Table 1A, compared with the control, the addition of three concentrations of faba bean stem and leaf extracts significantly inhibited the growth index of faba beans and was concentration dependent. Compared with the control, the exogenous addition of 0.01g·mL$^{-1}$ wheat stem and leaf extracts significantly increased the plant height, dry weight and root length of faba bean. Exogenously added 0.05 g·mL$^{-1}$ wheat stem and leaf extract slightly increased these parameters compared with the control. However, when the concentration of wheat stem and leaf extract reached 0.1 g·mL$^{-1}$, it significantly inhibited all the growth indices of faba bean (Fig. 3).

Table 1B shows that, compared with the control, the addition of 0.01 g·mL$^{-1}$ faba bean root extract significantly inhibited the main root length and root length of faba bean but had no significant effect on the other indicators. Compared with the control, the faba bean root extract with a concentration greater than or equal to 0.05 g·mL$^{-1}$ significantly inhibited all the growth indices of faba bean. In contrast, wheat

root extract had the opposite effect. Compared with the control, the addition of 0.01 g·mL$^{-1}$ wheat extract significantly increased all the growth indices of faba bean with the exception of number of leaves. The addition of 0.05 g·mL$^{-1}$ wheat root extract significantly increased the main root length, stem dry weight, root dry weight and root length of faba bean. However, when the concentration of wheat root extract reached 0.1 g·mL$^{-1}$, it significantly inhibited the plant height, main root length, stem weight, and root length of faba bean compared with the control and had no significant effect on the other indicators (Fig. 3).

The most notable effect is that the wheat extracts significantly increased the growth index of faba beans at three concentrations compared with the faba bean extracts.

Table 1

A. The effect of the extracts of faba bean and wheat stems and leaves on the growth of faba bean

| Aquatic extract from leaves and stem | Concentration (g·mL$^{-1}$) | Number of leaves per plant | Max leaf length (cm) | Plant height (cm) | Main root length (cm) | Shoot dry weight (g) | Root dry weight (g) | Root length (cm) |
|---|---|---|---|---|---|---|---|---|
| | CK | 10.00 ± 0.00a | 5.70 ± 0.60a | 22.87 ± 1.32b | 15.80 ± 0.78ab | 0.25 ± 0.06b | 0.17 ± 0.03b | 2.86 ± 0.14b |
| Faba bean | 0.01 | 8.00 ± 0.00bc | 4.9 ± 0.1b | 20.43 ± 1.40c | 10.60 ± 1.45c | 0.23 ± 0.03bc | 0.15 ± 0.01b | 2.41 ± 0.02c |
| | 0.05 | 7.33 ± 1.15 cd | 3.87 ± 0.06c | 16.3 ± 1.39d | 8.40 ± 0.66d | 0.15 ± 0.03d | 0.09 ± 0.01c | 1.45 ± 0.16d |
| | 0.1 | 6.00 ± 0.00d | 3.20 ± 0.56d | 10.3 ± 0.95e | 6.13 ± 0.60e | 0.07 ± 0.02e | 0.04 ± 0.02d | 0.39 ± 0.09e |
| Wheat | 0.01 | 10.67 ± 1.15a | 6.13 ± 0.31a | 26.33 ± 0.15a | 16.50 ± 0.92a | 0.36 ± 0.07a | 0.21 ± 0.04a | 3.51 ± 0.13a |
| | 0.05 | 9.33 ± 1.15ab | 5.80 ± 0.26a | 23.17 ± 1.29b | 13.70 ± 2.10b | 0.28 ± 0.01b | 0.14 ± 0.01b | 2.85 ± 0.16b |
| | 0.1 | 7.33 ± 1.15 cd | 4.63 ± 0.21b | 12.20 ± 0.53e | 6.90 ± 1.21de | 0.16 ± 0.05 cd | 0.11 ± 0.01c | 1.61 ± 0.12d |

The data is an average and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments ($P < 0.05$).

Table 1
B. The effect of the extracts of faba bean and wheat root on the growth of faba bean

| Aquatic extract from roots | Concentration ($g \cdot mL^{-1}$) | Number of leaves per plant | Max leaf length (cm) | Plant height (cm) | Main root length (cm) | Shoot dry weight (g) | Root dry weight (g) | Root length (cm) |
|---|---|---|---|---|---|---|---|---|
| | CK | 10.00 ± 0.00ab | 5.70 ± 0.60bc | 22.87 ± 1.32b | 15.80 ± 0.78b | 0.25 ± 0.06c | 0.17 ± 0.03 cd | 2.86 ± 0.14c |
| Faba bean | 0.01 | 9.33 ± 1.15bc | 5.57 ± 0.21bc | 22.60 ± 1.01b | 11.87 ± 1.67c | 0.23 ± 0.03 cd | 0.17 ± 0.02c | 2.54 ± 0.11d |
| | 0.05 | 8.00 ± 0.00 cd | 4.80 ± 0.10d | 17.83 ± 0.58c | 9.17 ± 0.49d | 0.16 ± 0.02e | 0.14 ± 0.01 cd | 1.72 ± 0.11e |
| | 0.1 | 6.67 ± 1.15d | 3.83 ± 0.35e | 10.50 ± 1.32e | 7.17 ± 0.25e | 0.07 ± 0.01f | 0.09 ± 0.03e | 0.97 ± 0.17f |
| Wheat | 0.01 | 11.33 ± 1.15a | 6.43 ± 0.35a | 26.90 ± 0.53a | 18.97 ± 0.59a | 0.39 ± 0.01a | 0.27 ± 0.01a | 3.69 ± 0.14a |
| | 0.05 | 10.00 ± 0.00ab | 5.93 ± 0.12ab | 23.53 ± 0.96b | 17.87 ± 0.31a | 0.32 ± 0.02b | 0.22 ± 0.04b | 3.19 ± 0.20b |
| | 0.1 | 8.67 ± 1.15bc | 5.33 ± 0.15 cd | 13.03 ± 0.59d | 11.87 ± 1.01c | 0.18 ± 0.06de | 0.12 ± 0.02de | 2.34 ± 0.13d |

The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments ($P < 0.05$).

## Effects of extracts from faba bean stems, leaves and roots on the physiological resistance of faba bean roots

A shown in Fig. 4A, compared with the control, the addition of 0.05 and 0.1 $g \cdot mL^{-1}$ faba bean stem and leaf extracts significantly reduced the POD activity of the faba bean root system. Compared with the faba bean stem and leaf extracts, the wheat stem and leaf extracts significantly increased the POD activity of the faba bean root in all concentrations tested. Figure 4B shows that the faba bean root extracts significantly reduced the POD activity of faba bean root in the 0.1 $g \cdot mL^{-1}$ treatment compared with that of the control. Compared with the faba bean root extracts, the wheat root extracts can significantly increase the POD activity of the faba bean root at all concentrations tested.

Figure 5A reveals that compared with the control, the faba bean stem and leaf extracts significantly inhibited the activity of CAT in the faba bean root system at 0.05 and 0.1 $g \cdot mL^{-1}$ concentrations. Compared with the faba bean stem and leaf extract, the wheat stem and leaf extracts can significantly increase the activity of CAT in the faba bean root system at all concentrations tested. As shown in Fig. 5B, the faba bean root extract at 0.05 and 0.1 $g \cdot mL^{-1}$ concentrations significantly inhibited the activity of CAT in the faba bean root system compared with that of the control. Compared with the faba bean root extract, the wheat root extract significantly increased the activity of CAT in the faba bean root at concentrations of 0.01 and 0.05 $g \cdot mL^{-1}$.

The effect of extracts from faba bean stems, leaves and roots on the MDA content of faba bean roots is shown in Fig. 6. The extracts of faba bean stems and leaves at all three concentrations significantly increased the content of MDA of faba bean roots compared with the control. This effect increases with concentration. Compared with the faba bean stem and leaf extracts, the wheat stem and leaf extracts in the three concentrations of treatment significantly reduced the content of MDA in the faba bean root system, and the effect was most significant in the 0.1 $g \cdot mL^{-1}$ treatment. As shown in Fig. 6B, compared with the control, 0.05 and 0.1 $g \cdot mL^{-1}$ of faba bean root extract significantly increased the content of MDA in the faba bean root system. Compared with the faba bean root extract, the wheat root extract at the three concentrations tested significantly reduced the MDA content in the faba bean root system, with the most significant effect visible at 0.1 $g \cdot mL^{-1}$.

## Effects of extracts from the leaves and stems and roots of faba bean and wheat on FOF spore germination and mycelial growth

As Fig. 7A indicates, compared with the control, the addition of 1.25, 5, 20, and 80 $mg \cdot L^{-1}$ faba bean stem and leaf extracts significantly inhibited the germination of FOF spores, but 640 $mg \cdot L^{-1}$ faba bean stem and leaf extracts significantly increased the germination of FOF spores. Compared with the treatment of faba bean stem and leaf extracts, the wheat stem and leaf extracts at concentrations of 1.25, 5, 20 and 80 $mg \cdot L^{-1}$ significantly inhibited the germination of FOF spores, with the strongest inhibitory effect at 5 $mg \cdot L^{-1}$ (Fig. 7A). Figure 7B shows that the faba bean root extracts significantly inhibited the germination of FOF spores at concentrations of 1.25, 5, 20 and 80 $mg \cdot L^{-1}$, but when the concentration reached 640 $mg \cdot L^{-1}$, the faba bean root extracts significantly promoted the germination of FOF spores. The wheat root extracts significantly inhibited the spore germination of FOF compared with faba bean root extracts when tested at 20, 80, 320 and 640 $mg \cdot L^{-1}$.

As Fig. 7C indicates, the extracts of faba bean stems and leaves significantly inhibited the mycelial growth of FOF at 6.25, 25 and 100 $mg \cdot L^{-1}$ concentrations compared with the control, and concentrations of 400, 800 and 1,600 $mg \cdot L^{-1}$ significantly stimulated the mycelial growth of FOF. Compared with the faba bean stem and leaf extracts, the wheat stem and leaf extracts significantly inhibited the mycelial growth of FOF in all concentrations. Figure 7D shows that the faba bean root extracts significantly inhibited the mycelial growth of FOF at concentrations of 25 and 100 $mg \cdot L^{-1}$, but they significantly

stimulated the mycelial growth of FOF at concentrations of 400, 800 and 1,600 mg·$L^{-1}$. Compared with the faba bean root extracts, the wheat root extracts significantly inhibited the mycelial growth of FOF at all concentrations tested.

# Discussion

Autotoxicity refers to the process by which plants or their residues release toxic chemicals into the environment during decomposition, thereby inhibiting the germination and growth of the same plant, and is a common cause of plant continuous cropping obstacles (Huang XX et al,2010). Currently, it has been proven that there are many autotoxic substances in plant root, stem and leaf extracts (J. C. Alías et al ,2006 ; Guo, K et al2016). This experiment showed that all concentrations tested of the extracts of faba bean stems, leaves, and roots significantly inhibited the growth of faba bean seedlings compared with the control, and the pronounced inhibition of root growth was particularly significant, which is similar to the results of Singh, N.B.et al (2008) on a study of tomato extracts. Plant cells accumulate free radicals owing to reduced antioxidant capacity during adverse conditions, leading to the oxidative damage of cellular macromolecules and membranes (Yin, Y.Q. et al, 2012). Furthermore, autotoxic metabolites produced by the stressed plants accelerate free radical-induced membrane peroxidation and breakdown, thereby providing nutrients to the pathogens and enhancing their ability to invade plant roots. In fact, the activity of the antioxidant enzymes POD and CAT are reliable indicators of disease resistance in plants (Ren LX. et al, 2008). Wang et al (2019) showed that exogenous syringic acid and phthalic acid significantly reduced the activities of POD and CAT in strawberry roots and increased the content of MDA. This is identical to the results obtained in this experiment. Compared with the control, medium and low concentrations of faba bean stem and leaves and root extracts significantly inhibited the activities of the antioxidant enzymes POD and CAT of the faba bean root system, while significantly enhancing the accumulation of MDA in faba bean roots. This could be because the extract of faba bean stems and leaves and roots contains a substantial amount of phenolic acids (Guo, K et al2016). They destroy the functional pathways of antioxidant enzymes and cause enormous damage to the defense system of faba bean roots, which in turn clears obstacles for the pathogens to invade faba bean roots. The accumulation of pathogen is the root cause of soil-borne diseases, and these microorganisms are difficult to remove from the soil. Experiments have proven that the three biological forms of *F. oxysporum* can survive for more than 11 years without changing their morphology (Sun, Y et al, 2017). Long-term continuous crops have formed a stable and suitable environment with increased temperature and humidity, sufficient nutrients and host conditions that are more conducive to the propagation and growth of pathogen, resulting in the aggravation of disease (Yang et al, 2001; Li et al, 2014a). In this experiment, the extracts of faba bean stems, leaves, and roots at low concentrations inhibited the spore germination and mycelial growth of FOF. However, with the increase in concentration, the inhibitory effect gradually disappeared, and at high concentration, the extracts significantly promoted germination and growth of the fungus. In actual agricultural production, owing to years of continuous cropping, the faba bean extracts had accumulated to large amounts in the soil, and the concentration of extract in the soil is very high. Therefore, in actual production, the faba bean extract has an enormous stimulatory effect on the

germination and growth of FOF. Based on these results, we hypothesize that the autotoxicity of faba bean may promote the growth of pathogen by destroying the defense system of faba bean root system and enhancing the invasion of pathogen to the root system of faba bean, finally resulting in strong inhibition of the growth of faba beans.

Intercropping is a green and efficient planting model, particularly in terms of increasing yield and controlling diseases. Now this advantage has been verified in many intercropping systems, such as corn and soybean intercropping that effectively controls corn crown rot and rice/watermelon intercropping that effectively controls watermelon wilt (Gao, X et al,, 2014; Ren LX et al, 2008). Similarly, we found that in field experiments, intercropping faba bean and wheat significantly inhibited the incidence of faba bean wilt in the faba bean branching and flowering stages, and during the branching, flowering and podding stages of faba bean, the disease index of faba bean wilt was significantly inhibited. Most research on the mechanism of intercropping disease control focuses on allelopathic substances secreted into the soil through the root system, but in actual production, these compounds can also enter the soil through the leaching and evaporation of plant roots, stems and leaves. These allelopathic substances are easily overlooked (Hao, Z. P et al, 2007). For the research on the mechanism of control by faba bean and wheat intercropping, we added different concentrations of wheat stem and leaf and root extracts in the faba bean hydroponic experiment. Compared with the faba bean extracts, we found that the wheat extracts significantly promoted the growth of faba bean seedlings at all treatment concentrations; similar conclusions have been obtained in the moringa and wheat intercropping system (Khan et al, 2017). We also simultaneously found that, compared with the treatment of faba bean extracts, wheat extracts significantly enhanced the activities of faba bean root POD and CAT and effectively reduced the accumulation of faba bean root MDA. The ability of the faba bean root system to resist the invasion of pathogen had improved. A series of results show that in actual production, wheat extracts can effectively alleviate the autotoxicity of faba beans. We hypothesize that this may be one of the important mechanisms of wheat and faba bean intercropping for disease control. This result is consistent with previous studies on rice/watermelon, rice/water chestnut, and corn/sunflower intercropping systems (Ren et al, 2008; Chen et al, 2012; Qin et al, 2013). On the basis of the significant improvement of the faba bean root defense system by wheat extracts, compared with the faba bean extracts, the wheat extracts could significantly inhibit the mycelial growth and germination of spores, thereby fundamentally reducing the possibility of pathogen infection of faba beans. This is consistent with the results of Ren et al (2010) on the wheat/watermelon intercropping system. This shows that in the wheat/faba bean intercropping system, the extracts of wheat can effectively relieve the stimulatory effects of the faba bean extracts on the occurrence of faba bean wilt, thereby further reducing the occurrence of faba bean wilt. Unexpectedly, compared with the control, the wheat extracts were effective at a low concentration, but they enhanced the inhibition of the growth of faba beans at high concentration. However, in actual production, unlike the large accumulation of faba bean extract, wheat has no continuous cropping history. The concentration of extract in the field is very low and is easily degraded by microorganisms in the soil. Therefore, in the actual field intercropping mode, the concentration of wheat extracts will not be very high. This experiment was a hydroponic one, and our aim was to explore the allelopathy of wheat extracts of different

concentrations. This does not examine the decomposition of allelochemicals by soil rhizosphere microorganisms. However, it also reminds us that in actual agricultural production, we should pay attention to controlling the ratio of faba bean and wheat and avoiding an excessive planting density of wheat that leads to an excessive concentration of rhizosphere wheat extract that could inhibit the growth of faba bean. After verification, we believe that the 2:6 ratio of faba bean:wheat used in this experiment is the best ratio to inhibit FOF, which is of substantial significance for the guidance of field production and disease control.

## Conclusions

In summary, wheat/faba bean intercropping can effectively control the occurrence of faba bean wilt. Studies on the extracts of faba beans and wheat found that the extracts of wheat improved the condition of faba bean seedlings, enhanced the physiological resistance of faba beans, eased the autotoxicity of faba beans and suppressed pathogenic fungal growth. All of these actions can reduce the occurrence of faba bean wilt under field conditions. This experiment should effectively guide the cultivation of faba bean and wheat intercropping in actual production. It can maximize the ability of faba bean and wheat intercropping to control disease and is highly significant to actual agricultural production. Although this is only preliminary research, it provides encouraging results and a basis for future research.

## Materials And Methods

# Test materials

The faba bean varieties (*Vicia faba* L.) used in this study, 89–147, and wheat (*Triticum aestivum* L.), Yunmai 53, were purchased from the Yunnan Academy of Agricultural Sciences (Kunming, China).

FOF was isolated from continuously cropped faba beans fields by the Plant-Microbe Laboratory at Yunnan Agricultural University, China. The fungus was transferred to potato dextrose agar (PDA) media, incubated at 28 °C for 7 days, and then stored at 4ºC.

# Field Trials

The field test was conducted in the experimental field of Hanbao, Kunming, Yunnan Province, China from October 2018 to May 2019. There was moderate rainfall during planting. The field lies in the humid subtropical zone and has a paddy soil type with topsoil (0 ~ 20 cm) that contained organic matter 14.5 g/kg, total nitrogen 1.21 g/kg, alkali nitrogen 59.8 mg/kg, available phosphorus 29.9 mg/kg, available potassium 52.1 mg/kg and had a pH of 6.5.

The faba beans were monocropped (MF) or intercropped with wheat (IF) in plots that measured 5.4 m × 6 m with a total area of 32.4 $m^2$. As shown in Fig. 1, the MF faba bean plants were sown at 0.1 m intervals, and the rows were spaced 0.3 m apart. Six rows of wheat and two rows of faba beans were

planted alternately in the IF plot for a total of three and four strips, respectively. The faba bean rows and intercropping faba bean and wheat rows were each spaced 0.3 m, whereas the wheat rows were spaced 0.2 m. The faba bean plants from the outermost rows of the 1st and 4th strips were not sampled. In addition, a 1 m-wide faba bean strip was planted around the entire test field as a protection line. Each treatment was repeated three times in six random blocks. No pesticides, fungicides or herbicides were applied throughout the growth period. Other management was conducted according to the local agronomic customs.

# Measurement Of The Incidence Of Fusarium Wilt

The severity of the disease was scored at different stages as: 0 – no symptoms of infection; 1 – slight plaques or discoloration at the base of the stem or peripheral roots; 2 – uneven lesions at the base of root or stem; 3 – uniform lesions, discoloration or wilting in 1/3 to 1/2 of the stem base or root and a reduction in lateral roots; 4 – completely discolored or withered roots or stem base, and 5 – complete wilting of the plant and death. The disease index and wilt incidence were calculated as:

$$\text{Incidence} = \frac{\text{Number of diseased plants}}{\text{total number of plants studied}} \times 100\%$$

$$\text{Disease index} = \frac{\Sigma(\text{Number of diseased plants at each level} \times \text{level})}{\text{The highest level} \times \text{total number of plants studied}} \times 100$$

# Preparation Of The Aqueous Extracts

At maturity, all the faba bean and wheat plants were collected from the experimental field, and the dust that adhered to the plant and root systems was rinsed with tap water and then deionized water. The plants were divided into two parts, roots and a combination of stems and leaves, which were desiccated in an oven at 105 °C for 30 min, dried at 65 °C to a constant weight and cut into small pieces 1 cm long. Twenty gram of dry samples of the roots, stems and leaves were weighed, respectively. A volume of 200 mL of deionized water was added and shaken at a constant temperature for 2 h. The solution was filtered through three layers of gauze after leaching at room temperature for 48 h and centrifuged at 4000 $r \cdot min^{-1}$ for 10 min. The supernatant was considered to be $0.1 g \cdot mL^{-1}$ plant water infusion mother liquor and stored at -20°C for use.

### Greenhouse cultivation

Faba bean seeds were soaked for 24 h at room temperature, germinated at 25 °C and sown in sterile quartz sand that had been soaked in Hoagland nutrient solution. Once the faba bean seedlings had

grown 4–6 leaves, they were transplanted into 2 L containers that contained various concentrations of aqueous extracts. The treatments included 0 (control), 0.01, 0.05 and 0.1 g·mL$^{-1}$ aqueous extracts. The controls treated by deionized water. There were three replicates for these treatments that resulted in 72 plants (three replicate pots × two types of extract × three seedlings × four concentrations).. The experiments were conducted under 24 h pump ventilation.

# Measurements Of Seedling Growth

The number of leaves per plant, maximum leaf length, height, main root length, shoot dry weight, and root dry weight were measured 30 days after transplantation.

# Evaluation Of Oxidative Stress Levels

POD activity was measured as previously described (Muñoz-Muñoz JL et.al, 2009; Quintanilla-Guerrero F et. al, 2008). Briefly, 1 g root samples were ground, and the homogenate was mixed with phosphate buffer. After centrifugation at 3000 rpm for 10 minutes, the supernatant was aspirated. A volume of 0.1 ml of the enzyme was mixed with 1 ml 2% $H_2O_2$, 2.9 ml 0.05 M phosphate buffer and 1 ml 0.05 M guaiacol in a 25 ml volumetric flask and incubated in 34 °C water for 3 min. The absorbance at 470 nm was measured every 5 minutes.

The activity of CAT was also measured as previously described (Manoranjan K. et al, 1976; Garcia-Limones C. et al, 2002). The root homogenate obtained as above was centrifuged for 15 minutes at 4000 rpm, and 2.5 ml of the supernatant and 0.1 M $H_2O_2$ were mixed and incubated for 10 min in a 30 °C water bath. After the addition of 2.5 ml 10% $H_2SO_4$, the solution was titrated with 0.1 M $KMnO_4$ until the solution turned pink. One unit of CAT is expressed as the number of milligrams of $H_2O_2$ decomposed in 1 minute per gram of fresh weight sample (mg · g$^{-1}$ · min$^{-1}$).

To measure the content of malondialdehyde (MDA), the end product of membrane lipid peroxidation (Bird BR, 1983), 0.5 g of the plant sample was homogenized in 5 ml of 5% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. The supernatant was aspirated, and 2 ml was boiled with the same volume of 0.67% thiobarbituric acid for 30 min, cooled and centrifuged. The absorbance was measured at 450, 532 and 600 nm.

# Evaluation Of Fof Growth And Conidial Germination

Mycelial discs that were 9 mm in diameter were plated onto PDA and cultivated at 28 °C for 7 days. The colony diameter was measured radially in three directions on days 3 and 7. A 9 mm agar plug was cut from the 7-day-old culture, inoculated into 15 ml PD media containing 0, 0.01, 0.05 or 0.1 g·mL$^{-1}$ faba bean or wheat aqueous extracts, and incubated for 7 days at 28℃ with constant shaking at 170 rpm.

The culture broth was filtered, dried at 80 °C for 12 h and weighed to determine the fungal biomass. The germination of spores was determined by washing the 7-day-old mycelia on PDA with sterile water and collecting the spores by filtration through four layers of gauze. The spore suspension was diluted to ≤ $1 \times 10^3$ CFU/ml, and 0.1 ml of spores were plated on each 2% (w/v) water agar plate containing 0, 0.01, 0.05 or 0.1 $g \cdot mL^{-1}$ faba bean or wheat aqueous extracts. The plates were incubated at 28°C for 3 days, and the number of colonies was counted. Each experiment was repeated in triplicate.

# Statistical analysis

The allelopathic effect of the extract was measured according to the response index (RI) proposed by Williamson and Richardson (1988).

All the data were analyzed using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA), and SPSS ver. 20.0 software (SPSS Inc., Chicago, IL, USA). The least significant difference (LSD) test was used to determine differences between the treatments at $P < 0.05$.

# Declarations

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### Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Availability of data and materials

All data generated or analysed during this study are included in this published article

## Competing interests

The authors declare that they have no competing interests

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## Authors' contributions

YG and JL completed the writing of this paper together

YD and KD co-directed the writing of the paper

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Not applicable

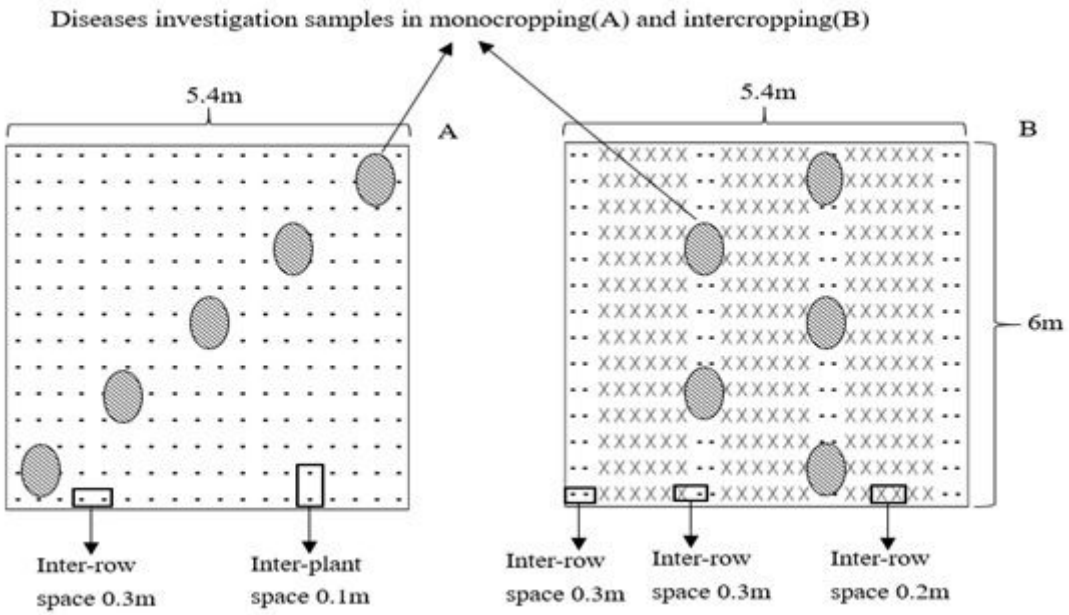
# References

1. Alghamdi SS, Migdadi HM, Ammar MH,et al. faba bean genomics: current status and future propects[J]. 2012,186(3):609–624.
2. Asaduzzaman M, Asao T. Autotoxicity in beans and their allelochemicals. entia Horticulturae. 2012;134(none):26–31.
3. Bird BR. Determination of malondialdehyde in biological materials by high-pressure liquid chromatography. analytical biochemistry. 1983;128:240–4.
4. Chen YG, Sui P, Luan C, Shi XP. Xanthium Suppression Under Maize||Sunflower Intercropping System. Journal of Integrative Agriculture. 2012;6(11):1026–37.
5. Eldarier SM, Eldien MH. Biological activity of Medicago sativa L. (alfalfa) residues on germination efficiency, growth and nutrient uptake of Lycopersicon esculentum L. (tomato) seedlings. Journal of Taibah University for Science. 2011;5(1):7–13.
6. Gao X, Wu M, Xu R, Wang X, Pan R, Kim H, Liao H. (2014). Root Interactions in a Maize/Soybean Intercropping System Control Soybean Soil-Borne Disease, Red Crown Rot. PLOS ONE, 9(5).
7. Garcia-Limones C, Hervas A, Navas-Cortes JA, Jimenez-Diaz RM, Tena M. Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (Cicer arietinum L.) and Fusarium oxysporum f.sp.ciceris. Physiol Mol Plant Pathol. 2002;61:325–37.
8. Gil VS, Haro R, Oddino C, Kearney M, Zuza M, Marinelli A, March GJ. Crop management practices in the control of peanut diseases caused by soilborne fungi. Crop Prot. 2008;27:1–9.
9. Grodzinsky AM. Allelopathic effects of cruciferous plants in crop rotation. In: Rizvi SJH, Rizvi V, editors. Allelopathy: Basic and Applied Aspects. London: Chapman& Hall; 1992. pp. 77–86.
10. Guo K, He X, Yan Z, Li X, Qin B.. (2016). Allelochemicals from the rhizosphere soil of cultivated astragalus hoantchy. J Agric Food Chem, 64(17).
11. 10.1016/j.scientia.2006.12.030
    Hanwen Wu J, Pratley D, Lemerle M, An, De Li L. (2007). Autotoxicity of wheat (triticum aestivum l.) as determined by laboratory bioassays. plant & soil, 296(1–2), 85–93.Hao, Wang ZP, Christiea Q, P., & Li XL. (2006). Allelopathic potential of watermelon tissues and root exudates. Scientia Horticulturae. DOI: 10.1016/j.scientia.2006.12.030.
12. Hao WY, Ren LX, Ran W, Shen QR. Allelopathic effects of root exudates from watermelon and rice plants on fusarium oxysporum f.sp. niveum. Plant Soil. 2010;336(1–2):485–97.

13. Hao ZP, Wang Q, Christie P, Li X. Allelopathic potential of watermelon tissues and root exudates. Sci Hortic. 2007;112(3):315–20.
14. Huifang L, Haishun C, Nawaz MA, Hamza S, Yuan H, Fei C, et al. Wheat intercropping enhances the resistance of watermelon to fusarium wilt. Frontiers in Plant ence. 2018;9:696.
15. Alías JC, Sosa T, Escudero JC, Chaves N. (2006). Autotoxicity against germination and seedling emergence in cistus ladanifer l. Plant & Soil, 282(1–2), 327–332.Li X, Zhang Y, Ding C, Xu W, Wang X. (2017). Temporal patterns of cotton Fusarium and Verticillium wilt in Jiangsu coastal areas of China. Scientific Reports, 7(1), 1–8.
16. Khan S, Basra SMA, Afzal I, Nawaz M, Rehman HU. Growth promoting potential of fresh and stored Moringa oleifera leaf extracts in improving seedling vigor, growth and productivity of wheat crop. Environ Sci Pollut Res. 2017;24:27601–12.
17. Li X, De Boer W, Zhang YN, Ding C, Zhang T, Wang X. Suppression of soil-borne Fusarium pathogens of peanut by intercropping with the medicinal herb Atractylodes lancea. Soil Biol Biochem. 2018;116:120–30.
18. Li X, Zhang Y, Ding C, Xu W, Wang X. Temporal patterns of cotton fusarium and verticillium wilt in jiangsu coastal areas of china. Sci Rep. 2017;7(1):12581.
19. Li XG, Ding CF, Hua K, Zhang TL, Zhang YN, Zhao L, Yang RY, Liu YG, Wang XX. Soil sickness of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to direct allelopathy. Soil Biology Biochemistry. 2014a;78:149–59.
20. Li XG, Wang XX, Dai CC, Zhang TL, Xie XG, Ding CF, et al. Effects of intercropping with atractylodes lancea and application of bio-organic fertiliser on soil invertebrates, disease control and peanut productivity in continuous peanut cropping field in subtropical china. Agrofor Syst. 2014;88(1):41–52.
21. Manoranjan K, Dinabandhu M. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol. 1976;57:315–9.
22. Minuto A, Davide S, Garibaldi A, Gullino ML. Control of soil-borne pathogens of tomato using a commercial for mulation of Streptomyces griseoviridis and solarization. Crop Prot. 2006;25:468–75.
23. Muñoz-Muñoz JL, García-Molina F, García-Ruiz PA, Arribas E, Tudela J, García-Cánovas F, Rodríguez-López JN. Enzymatic and chemical oxidation of trihydroxylated phenols. Food Chem. 2009;113(2):435–44.
24. Ohno S, Tomita-Yokotani K, Kosemura S, Node M, Suzuki T, Amano M, Yasui K, Goto T, Yamamura S, Hasegawa K. A species-selective allelopathic substance from germinating sunflflower (Helianthus annuus L.) seeds. Phytochemistry. 2001;56:577581.
25. Qin JH, He HZ, Luo SM, Li HS. Effects of rice-water chestnut intercropping on rice sheath blight and rice blast diseases. Crop Protection. 2013;43:89–93.
26. Quintanilla-Guerrero F, Duarte-Vázquez MA, García-Almendarez BE, Tinoco R, Vazquez-Duhalt R, Regalado C. Polyethylene glycol improves phenol removal by immobilized turnip peroxidase. Biores Technol. 2008;99(18):8605–11.

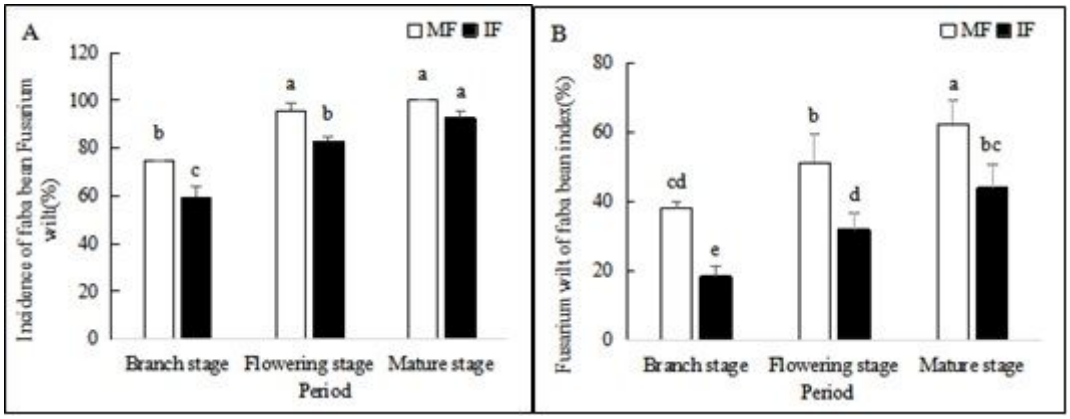
27. Ren L, Huo H, Zhang F, Hao W, Xiao L, Dong C, et al. (2016). The components of rice and watermelon root exudates and their effects on pathogenic fungus and watermelon defense. plant signaling & behavior.
28. Ren LX, Su SM, Yang XM, Xu YC, Huang QW, Shen QR. Intercropping with aerobic rice suppressed Fusarium wilt in watermelon. Soil Biol Biochem. 2008;40:834–44.
29. Stoddard FL, Nicholas AH, Rubiales D, Thomas J, Villegas-Fernández AM. Integrated pest management in faba bean. Field crops research. 2010;115(3):308–18.
30. Sun Y, Wang Y, Han LR, Zhang X, Feng JT. (2017). Antifungal Activity and Action Mode of Cuminic Acid from the Seeds of Cuminum cyminum L. against Fusarium oxysporum f. sp. Niveum (FON) Causing Fusarium Wilt on Watermelon. Molecules, 22(12).
31. Sunaina, Singh NB. Alleviation of allelopathic stress of benzoic acid by indole acetic acid in solanum lycopersicum. entia Horticulturae. 2015;192:211–7.
32. Tesio F, Vidotto F, Ferrero A. Allelopathic persistence of helianthus tuberosus l. residues in the soil. entia Horticulturae. 2012;135(none):98–105.
33. Wang XQ, Du GD, Lu XF, Ma HY, Lyu DG, Zhang H, Song JL. Characteristics of mitochondrial membrane functions and antioxidant enzyme activities in strawberry roots under exogenous phenolic acid stress. Sci Hort. 2019;248:89–97.
34. Williamson GB, Richardson D. Bioassays for allelopathy: measuring treatment responses with independent controls. Journal of Chemical Ecology. 1988;14(1):181–7.
35. Xiao-Gang L, Tao-Lin Z, Xing-Xiang W, Ke H, Ling Z, Zheng-Min H. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. International Journal of Biological ences. 2013;9(2):164–73.
36. Xingxue H, Zhilong, Bie Y, Huang. (2010). Identification of autotoxins in rhizosphere soils under the continuous cropping of cowpea. Allelopathy Journal.
37. Yang CH, Crowley DE, Menge JA. 16S rDNA fingerprinting of rhizospherebacterial communities associated with healthy and Phytophthora infected avocado roots. FEMS Microbiology Ecology. 2001;35(2):129–36.
38. Ye SF, Zhou YH, Sun Y, Zou LY, Yu JQ. Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of Fusarium wilt. Environ Exp Bot. 2006;56:255–62.
39. Yin YQ, Hu JB, Deng MJ. Latest development of antioxidant system and responses to stress in plant leaves. Chin Agric Sci Bull. 2012;23(1):105–10.
40. Young CC. 1984. Autotoxication in root exudates of Asparagus offificinalis L. PlantSoil 82, 247–253.
41. Yu JQ, Su FY, Ming FZ. (2003). Effects of root exudates and aqueous root exudates of cucumber (Cucumis sativus) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochemistry, Systematic and Ecology, 31, 129139.

# Figures




## Figure 1

Diagram of the planting patterns in the field experiments: (A) the monocroppingfaba bean plot, (B) the intercropping plot of faba bean with wheat. - , faba bean; ×, wheat.

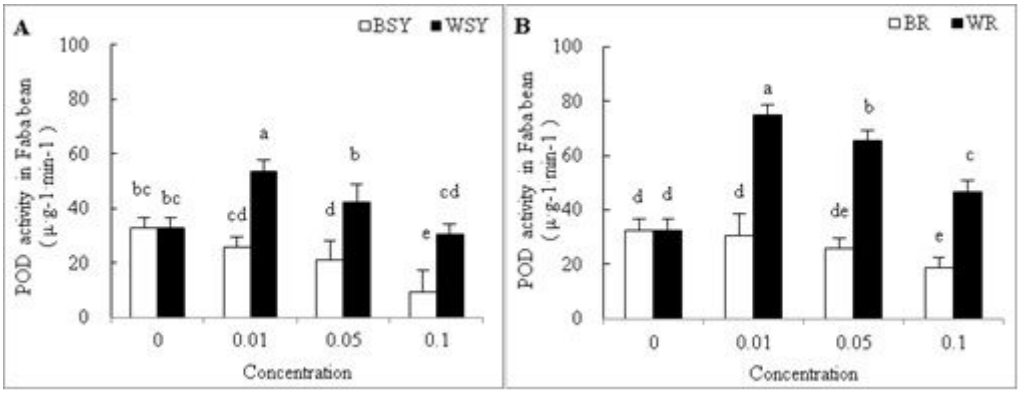



## Figure 2

The effect of wheat and faba bean intercropping on faba bean wilt; (A) incidence of faba bean wilt; (B) faba bean wilt disease index. The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments ($P<0.05$).

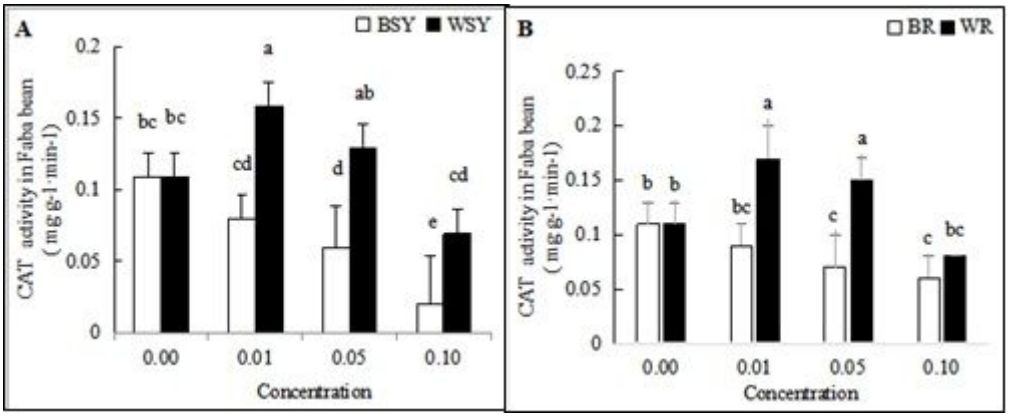

## Figure 3

Growth of the faba beans under different treatments. BR: treatment with exogenously added faba bean root extract; BSY: treatment with exogenously added faba bean stem and leaf extract; WR: treatment with exogenously added wheat root extract; WSY: treatment with exogenously added wheat stem and leaf extract. 1, 2 and 3 represent concentration gradients of 0.01, 0.05 and 0.1 g•mL-1 of wheat stem and leaf extracts, respectively.



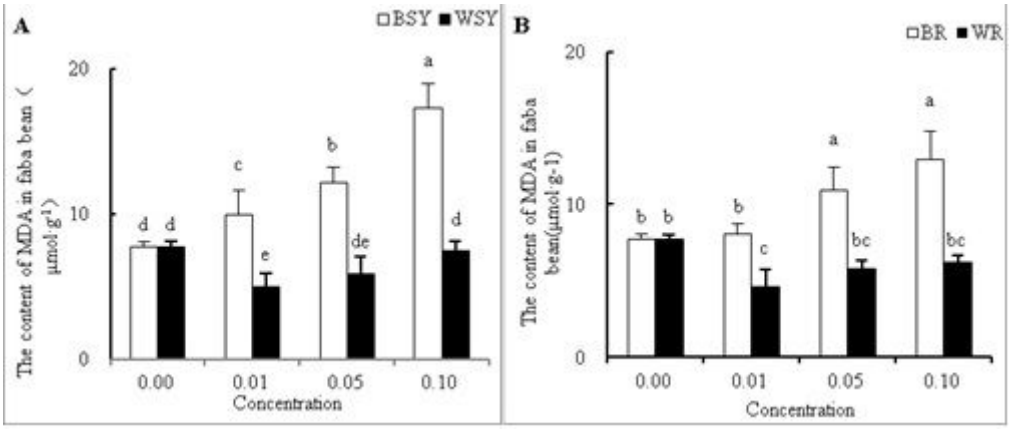
## Figure 4

Effect of extracts from faba bean stems, leaves and roots on POD activity of faba bean roots.BR: treatment with exogenously added faba bean root extract; BSY: treatment with exogenously added faba bean stem and leaf extract; WR: treatment with exogenously added wheat root extract; WSY: treatment with exogenously added wheat stem and leaf extract. The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments (P<0.05).




## Figure 5

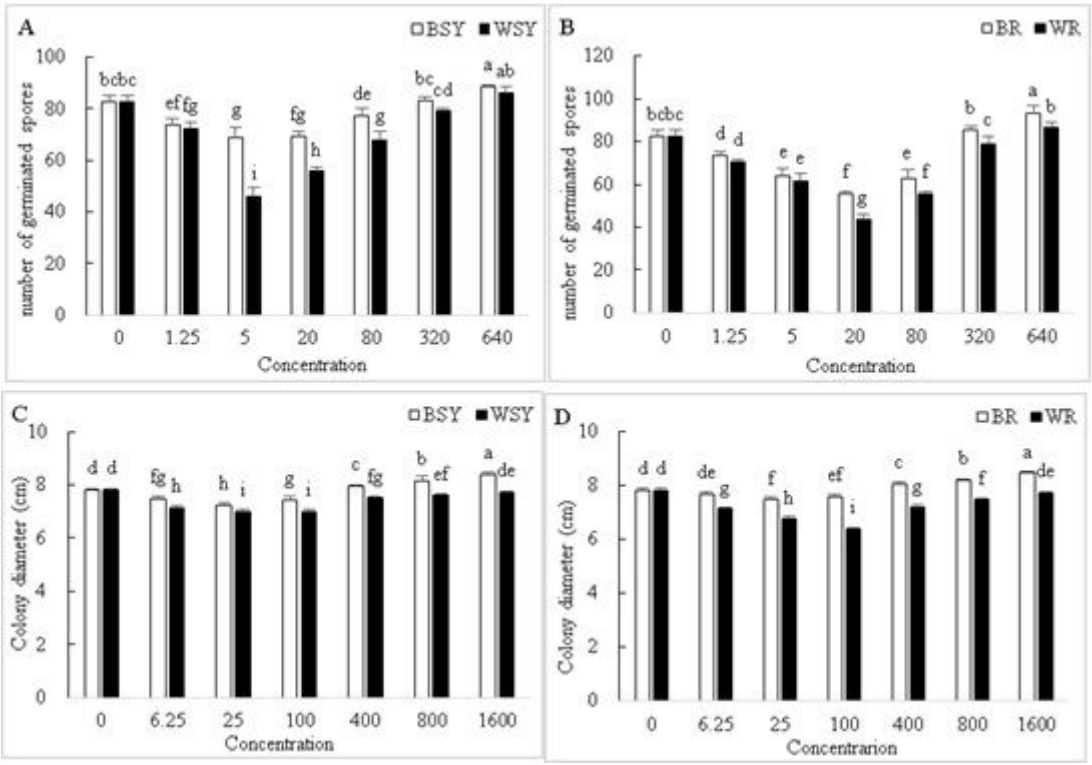
The effects of extracts from faba bean stems, leaves and roots on CAT activity of faba bean roots.BR: treatment with exogenously added faba bean root extract; BSY: treatment with exogenously added faba bean stem and leaf extract; WR: treatment with exogenously added wheat root extract; WSY: treatment with exogenously added wheat. The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments (P<0.05).




## Figure 6

Effects of extracts from faba bean stems, leaves and roots on the MDA content of faba bean roots.BR: treatment with exogenously added faba bean root extract; BSY: treatment with exogenously added faba bean stem and leaf extract; WR: treatment with exogenously added wheat root extract; WSY: treatment

with exogenously added wheat stem and leaf extract. The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments (P<0.05).




## Figure 7

(A) Effect of faba bean and wheat stem and leaf extracts on the germination of FOF spores; (B) faba bean and wheat root extracts on the germination of FOF spores; (C) faba bean and wheat stem and leaf extracts on FOF mycelium growth effect; (D) The effect of faba bean and wheat root extract on FOF mycelial growth.BR: treatment with exogenously added faba bean root extract; BSY: treatment with exogenously added faba bean stem and leaf extract; WR: treatment with exogenously added wheat root extract; WSY: treatment with exogenously added wheat stem and leaf extract. The data is an average, and the standard error of three repetitions is represented by a bar. Different letters in the figure indicate significant differences between different treatments (P<0.05).