ANSF
APPLIED AND NATURAL SCIENCE FOUNDATION

Journal of Applied and Natural Science
15(4), 1499 - 1504 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Estimation of phenyl alanine ammonialyase (PAL) and some phytoalexins in *Vicia faba* plants infected with spot disease caused by *Alternaria alternata*

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Article Info
https://doi.org/10.31018/jans.v15i4.4811
Received: September 12, 2023
Revised: November 26, 2023
Accepted: December 2, 2023

How to Cite

Al-Ameri, H. A. (2023). Estimation of phenyl alanine ammonialyase (PAL) and some phytoalexins in *Vicia faba* plants infected with spot disease caused by *Alternaria alternata*. *Journal of Applied and Natural Science*, 15(4), 1499 - 1504. https://doi.org/10.31018/jans.v15i4.4811

Abstract

Biological agents are an important approach to controlling many fungal phytopathogens; plants produce phytoalexins such as phenol, alkaloids and tannins as a response to the biotic stresses. The results showed that the addition of the biological control of *Trichoderma harzianum* to *Alternaria alternata* caused an increase in the activity of the enzyme phenylalanine ammonialyase (PAL) after 20 days from the start of the greenhouse experiment, which was 6.21 mmol/min in the Spinal variety, compared with the enzyme activity in the treatment with *A. alternata* alone, which was 5.51 mmol/min. The variety Netherland mulch recorded a lower phenolic content in all treatments, and the treatment with biological control *T. harzianum* in addition to *A. alternata* had the highest content as it reached 4.50 mg/g on day 20, Netherland mulch variety showed the most amount of alkaloids and tannins than others during different periods. The plants treated with the biological control of *T. harzianum* contained the most alkaloids on day 20, which was 5.15% It decreased to 3.65% on day 30. The most tannins in Netherland mulch variety were in the plants treated with biological control *T. harzianum* alone, which was 3.93% on day 20, compared to the plants treated with *A. alternata* alone, which was 0.71%. The addition of biological control of *T. harzianum* to *A. alternata* increased the plant amount of tannins, which was 2.01%. The study would help to understand the role of *T. harzianum* as a biological control to the response of the varieties to the formation of plant defenses, including phytoalexins.

Keywords: Alkaloid, *Alternaria alternata*, PAL, phenol, *Trichoderma harzianum*, Tannins, *Vicia faba*

INTRODUCTION

Bean (*Vicia faba* L.) is a common crop farmed for food and fodder; its seeds are a reliable energy source fiber and protein. The seed dry matter content protein is 24–35% of an average of 29% (Crépon *et al*., 2010), making it the most leguminous crop rich in protein (Robinson *et al*., 2019). It contains a high percentage of essential amino acids and lysine in the diet, a good protein source (Khazaei and Vandenberg, 2020), and contains polyphenols, which are antioxidants. Eating legumes helps reduce heart disease cancer (Crépon *et al*., 2010; Khazaei and Vandenberg, 2020). Many fungal pathogens infected *Vicia faba* plant, causing great losses in the crop, as it was attacked by many foliar diseases such as chocolate spot caused by *Botrytis fabae* and *B. cinerea*, rust caused by *Uromyces fabae*, downy mildew caused by *Peronospora viciae*. *Alternaria alternata* was identified as the cause of *Alternaria* leaf spot. This fungus is characterized by its ability to produce different types of toxins that contribute to the invasion of plant tissue and infection and its carcinogenic effects on human cells (Reis *et al*., 2007; Juroszek *et al*., 2011).

Various control methods, including biological and chemical methods, cross-prevention, agronomic resistance and practices varieties, were studied to control *A. alternata*, causing faba bean spotting leaf. Sometimes, chemical control against this pathogen gave good results, but improper use of fungicides often leads to resistance to *A. alternata* and risks of environmental contamination (Kamble *et al*., 2000; Wang *et al*., 2022) Biological agents are an important approach to control many fungal phytopathogens and exploration of new biological agents as antibiotics for biological control is also increasing, Inhibition of growth of *A. alternata* that causes bean leaf spot disease by *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride* (Kumar *et al*., 2020).

The mechanisms of activating resistance in plants depend on the presence of the pathogen. These mechanisms include increased peroxidase activity, β-1,3-glucanase, chitinase, phenylalanine ammonia-lyase and polyphenol oxidase, leading to systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Chen *et al*., 2017). Phenyl alanine ammonialyase (PAL) enzyme is an essential enzyme in secondary metabolites of plant biosynthesis, such as the production of many phenolic compounds that lead to an increase in lignin deposition and the thickness of cell walls. Entering the second pathway, phenylpropanoid, which converts phenylalanine into trans-cinnamic acid, and the latter is converted into many phenolic compounds necessary for physiological processes, plant growth and development, and interactions between the environment and plants, especially resistance to pathogens such as lignin precipitation and structural strengthening (Zhang and Chang-Jun, 2015; Cass *et al*., 2015 and Mssillou *et al*., 2022).

Phenols are secondary metabolic products in plants that arise from the shikimic acid and malonic acid pathways. They are a large group of compounds that are divided into groups: Coumarin, Lignin, Flavonoids, Phenolic acids, and Tannins. They are necessary for the physiological processes of the plant and the interactions between the environment and the plant, and they have a role as defense compounds for pathogens and act as powerful antioxidants (Lattanzio, 2013; Małolepsza *et al*., 2017).

Phytoalexins are antimicrobial compounds with low molecular weight produced by plants in response to abiotic and biotic stresses. The rapid accumulation of phytoalexins after infection gives a positive result on plant resistance to pathogens, accumulates at the site of infection, inhibits the radial growth of the fungus, and inhibits bacterial tube elongation and asymmetric growth (Singh and Chandrawat, 2017; Sen, 2017).

The aim of the present study was an estimation of phenylalanine ammonialyase (PAL) and some phytoalexins such as total phenol content, alkaloid and tannins in three varieties of *V. faba* plants infected with *A. alternata* after 10, 20 and 30 days of adding the pathogen *A. alternata* and study the effect of *T. harzianum* as a biological control in the content of these parameters.

MATERIALS AND METHODS

Pathogenic fungi used in treatments

Alternaria alternata isolated from the leaves of *Vicia faba* plants infected with spotting were cut into pieces of 1 cm and superficially sterilized with 70% ethanol for 3 minutes, then washed with sterile distilled water, dried with sterile filter paper and transferred by sterile forceps to a Petri dish containing Potato Dextrose Agar (PDA) medium with the antibiotic Streptomycin at a concentration of 50 Mg/ml. The plates were incubated at a temperature of 28^o C for 7 days. The fungus was identified using taxonomic keys (Ellis, 1971; Barnett and Hunter, 2006 ; Cass *et al*., 2015 ; Basım *et al*., 2018).

Varieties *of Vicia faba* used in treatments

Three *V. faba* varieties were used in treatments: Netherland mulch, Local Syria and spinach.

Biological control used in treatments

Trichoderma harzianum was the biological control used in treatments.

Treatments in greenhouse

The three soil treatments:1. Soil treatment with *A. alternata* alone, 2. Soil treatment with *A. alternata* + *T. harzianum* and 3. Soil treatment with *T. harzianum* alone and 4- Sterile soil (control). According to Saydam *et al*. (1973), the greenhouse treatments were carried out.

Estimation of the activity of the PAL enzyme

0.5 g. of leaflets were taken for each treatment and crushed with 2 ml of phosphate buffer pH 6 (buffer was prepared by mixing a solution of NaH_2PO_4 and Na_2HPO_4 at a concentration of 0.1 M for each) and placed the mixture in test tubes of 10 ml, the tubes underwent a rapid centrifugation process 4000 rpm for 15 minutes, and this was the supernatant (filtrate solution). Spectrophotometer type (PHILIPS – PYE UNICAM SP6 -350) was used to estimation of the activity of APL by added 0.5 ml of the phenylalanine (amino acid as substrate) at a concentration of 6 μM with 0.5 ml of Tris-HCl solution at a concentration of 0.5 M pH 8 with 0.2 ml of the filtrate solution in test tubes, incubating the mixture for 4 h. at a temperature of 37^{o}C The reaction was stopped by adding 0.05 ml of HCl at a concentration of 5 M. Then the color change was measured at 290 nm for three replicates of each treatment (Beavdoin-Eagan and Thorpe, 1985), after 10, 20 and 20 days of adding the pathogen *A. alternata*.

Estimation of phytoalexins

Estimation of total phenol content in the plants

Phenols were calculated based on mg of phenols/g of plant tissue and used catechol (standard) after 10, 20 and 20 days of adding the pathogen (Rishi *et al*., 2008). 1 g. of fresh plant leaves were with methanol (10 ml) at a concentration of 80% and placed in a water bath at 70^o C with continuous stirring for 15 min. After filtration, 5 ml of distilled water was mixed with 1 ml of the filtrate and 2.5 ml of Follin's reagent in a tube and incubated at 25 ^{o} C for 30 minutes. An appropriate amount of the solution was taken and placed in the device's cell. The Spectral absorbance was measured at 725 nm.

Estimation of the alkaloid content in the plants

Five gm. of dry bean leaves were taken from all treatments for the three varieties and placed in a beaker and 200 ml of acetic acid at a concentration of 10% was added to it (10 ml of acetic acid 90 ml of ethanol). The samples were placed for 4 hours in a water bath. The solution was filtered and concentrated to a quarter. The original volume and a drop of NH4OH were added until sedimentation was completed. Then, the precipitate was taken, washed with a dilute solution of NH_4OH and filtered afterwards. Then, the precipitate representing the alkaloids was taken, dried and weighed, and the percentage was calculated on the basis of the dry weight (Abbas *et al*., 2012).

Estimation of tannins content in the plants

The method of Boham and Kocipai-Abyazan (1994) was adopted. 0.5 gm. of dry bean leaves were taken from all treatments for the three varieties, and 75 ml of distilled water was added to it and placed in a boiling water bath for 30 minutes, then centrifuged at a speed of 2000. The filtrate was placed in a beaker and made up to 100 ml with distilled water. Then 20 ml of lead acetate at a concentration of 4% was added, placed in a shaking device for an hour, and filtered with filter paper. The precipitate was taken and dried in an oven at 105 °C for an hour. The weight of the ceramic eyelid was taken, heated at 55°C, weighing the eyelid again, and calculating the percentage of tannins was according to the equation no. 1 below:

% Tannins = AX B/ C X100 Eq.1

A: Weight of the residue before burning; B: Weight of the residue after burning; C: Sample weight

RESULTS

Activity of the PAL enzyme

The addition of the biological control *T. harzianum* to *A. alternata* caused an increase in the activity of the enzyme PAL after 20 days from the start of the greenhouse experiment, which was 6.21 mmol/min in the Spinal variety, compared with the enzyme activity in the treatment with *A. alternata* alone, which was 5.51 mmol/min, as well as the treatment in sterile soil (control) in which it reached the activity of the enzyme was 4.32 mmol/min, and the highest activity of the enzyme was in the treatment of biological control *T. harzianum* alone, which was 6.97 mmol/min.

Netherland mulch variety showed different effects. The addition of biological control *T. harzianum* to *A. alternata* gave the highest activity of the enzyme on day 20, reaching 5.72 mmol/min, and it decreased on day 30 until it remained higher than its effectiveness on day 10. Local Syria variety was the least of the varieties responding to the biological control, as the activity of the enzyme in the treatments of biological control *T. harzianum* added to *A. alternate* was 5.17 mmol/min on the day 20 compared to treatment with *A. alternata* alone, as the activity of the enzyme PAL reached 3.58 mmol/min and decreased on the day 30. It remained higher than the enzyme activity on day 10, and the enzyme activity in the control treatment (sterilized soil) was 2.43 mmol/min, 1.22 mmol/min, and 1.01 mmol/min on days 20, 30, and 10, respectively (Fig. 1).

Estimation of total phenol content in the plants

The results in Fig. 2 show that the addition of the biological agent to the fungus led to an increase in the total phenols in treated plants, and it reached its highest level on day 20 of the start of the greenhouse experiment, then it began to decrease on day 30. However, it remained higher than the day 10 in all the studied varieties. Spinal variety plants were the highest in the content of total phenols, and the treatment with biological agent *T. harzianum* added to *A. alternata* recorded an amount of 5.21 mg/g, compared to the treatment with *A. alternata* alone, as the amount of total phenols was 4.04 mg/g. The treatment with biological control *T. harzianum* alone had the highest amount of phenols, reaching 5.92 mg/g on day 20. The total phenols decreased in all treatments on day 30 until it remained higher than its amount on day 10.

In all treatments, the variety Netherland mulch recorded a lower phenolic content. The treatment with biological control *T. harzianum,* in addition to *A. alternata* had the highest content as it reached 4.50 mg/g on day 20. It decreased on day 30 and amounted to 3.62 mg/g. It differed from the local Syria variety in the studied periods and was the lowest in total phenols content.

Estimation of the alkaloid and tannins percentage in the plants

Netherland mulch variety had the most amount of alkaloids and tannins than others during different periods. The plants treated with the biological control *T. harzianum* had the alkaloids highest amount of on day 20, which was 5.15%. It decreased to 3.65% on day 30 but remained higher than its amount on day, which was 10 3.11% compared to plants treated with *A. alternata* alone, which was 1.31% on day 20 and decreased to 0.75% on day 30. There was no amount of alkaloids on day 10. The addition of biological control *T. harzianum* to *A. alternata* increased the amount of alkaloids, which was 3.92% on day 20. The amount of alkaloids in the treated plants of Local Syria and Spinal varieties differed according to the treatments compared to the control treatment (sterilized soil).

As for the plant amount of tannins, it differed according to different treatments and varieties. The most tannins

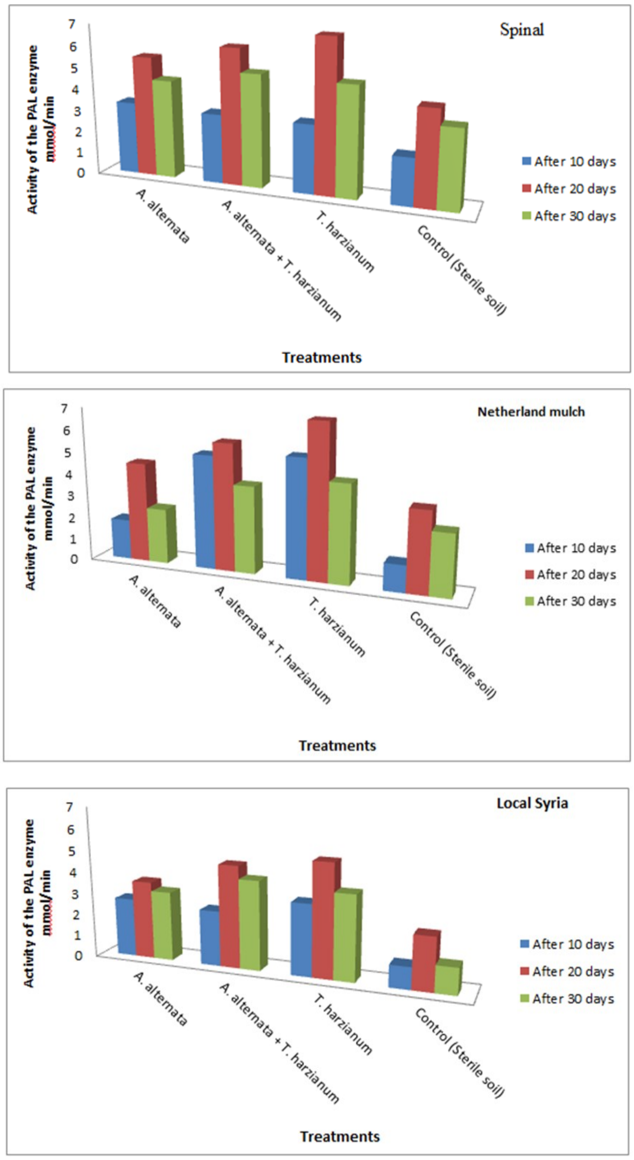

Fig. 1. *Effect of different treatments on the activity of the PAL enzyme (mmol/min) for Vicia faba varieties plants*

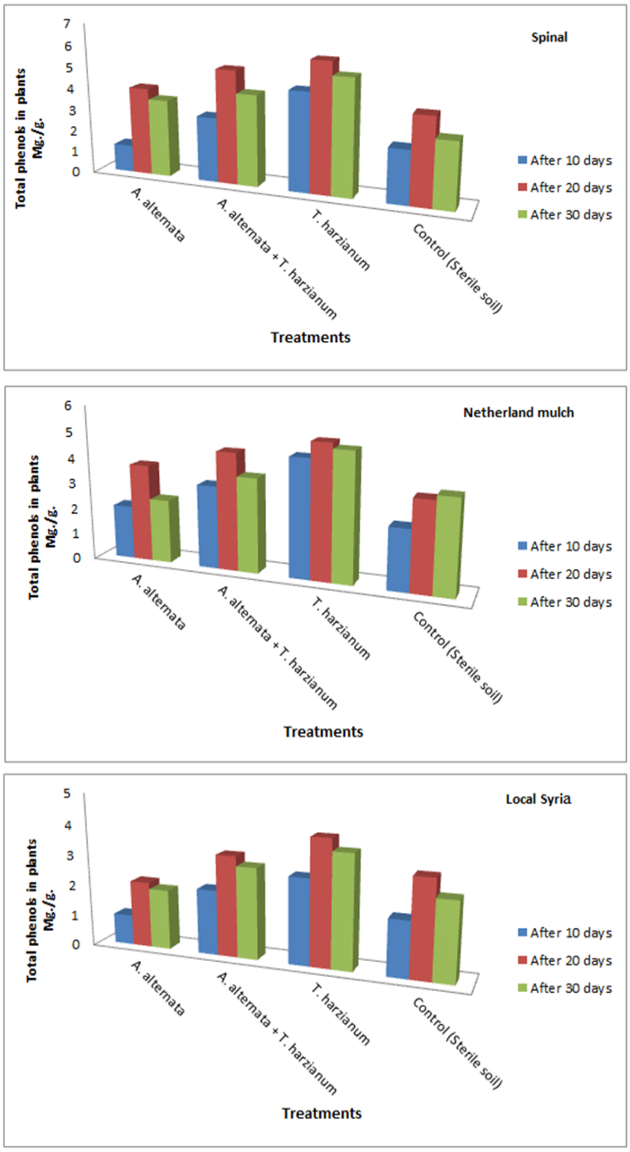

Fig. 2. *Effect of different treatments on total phenols for Vicia faba varieties plants*

amount in Netherland mulch variety was in the plants treated with biological control *T. harzianum* alone, which was 3.93% on day 20, compared to the plants treated with *A. alternata* alone, which was 0.71%. The addition of biological control *T. harzianum* to *A. alternata* increased the plant amount of tannins which was 2.01%, and tannins amount in the plants treated in Local Syria and Spinal varieties differed with the different treatments compared to the control treatment (Table 1).

DISCUSSION

Induced systemic resistance is defined as an increase in the plant's defensive ability against pathogens after stimulating the defense means by the induced (Mhlongo *et al*., 2018). It is a set of structural and chemical reactions that fail or hinder infection (Sharma *et al*., 2018). PAL works in the biosynthesis of secondary metabolites, such as producing many phenolic compounds, which lead to increased lignin precipitation and cell wall thickness (Chen *et al*., 2017; Khazaei *et al*., 2019).

PAL is the first enzyme to work through the phenylpropanoid produced in the biological structure that regulates various metabolic processes such as Coumaric acid, Cinnamic acid, Ferulic acid, Caffeic acid, flavonoids, tannins and lignin. These products protect the plant against various abiotic stresses and pathogens that attack them as evidence of positive defense (Hahlborck and Sheel, 1989).

T. harzianum has a role in catalyzing and activating4-cinnamate hydroxylase, 4-Coumarate CoA ligase and PAL, causing an increase in the activity of these

Table 1. Effect of different treatments on the percentage of alkaloids and tannins for the *Vicia faba* varieties plants

Type of phytoalexins	Treatments	Varieties								
		Netherland mulch			Local Syria			Spinal		
		Days								
		10	20	30	10	20	30	10	20	30
Alkaloids	*A. alternata*	-	1.31	0.75	-	0.53	-	-	1.2	0.93
	A. alternata + *T. harzianum*	1.24	3.92	2.41	1.20	1.97	1.51	1.15	3.75	2.65
	T. harzianum	2.11	5.15	3.65	2.76	3.35	3.52	1.42	3.9	2.93
	Control (Sterile soil)	1.89	2.84	2.0	0.10	0.72	0.21	0.54	2.99	2.72
Tannins	*A. alternata*	0.21	0.71	0.53	-	0.2	-	-	0.52	0.30
	A. alternata + *T. harzianum*	1.23	2.01	1.73	0.1	0.95	0.34	0.32	1.32	0.71
	T. harzianum	1.54	3.93	2.54	0.35	1.21	1.0	0.37	2.01	0.95
	Control (Sterile soil)	0.54	1.75	0.93	0.14	0.72	0.54	0.01	0.99	0.30

enzymes and many compounds accumulation that decreased microorganisms growth, including acid, Salicylic Acid, Benzoic Acid, and Ethylene, Derivatives of Jasmonic Acid, PAL, after induction by a living or abiotic agent, works by building lignin primers and other cyclic compounds and is itself toxic for pathogenic organisms such as Phytoalexin compounds (Gveroska. and Jovancev, 2011), these precursors condense in the Shikimic Acid Pathway to form Lacticin, which is the most complex organic compound on the face of nature (Al-Ameri; Ramadan, 2022). This is confirmed by the results shown in Fig. 1. The highest activity of PAL enzyme was in the treatment of biological control *T. harzianum* alone. The addition of the biological control *T. harzianum* to *A. alternata* caused an increase in the activity of the enzyme PAL, and phytoalexins which are total phenol, as shown in Fig. 2 and the percentage of alkaloids and tannins for the *Vicia faba* varieties plants as in Table 1 as compared with the treatment with *A. alternata* alone, The reason for the difference in the effect of the biological control on the PAL activity and phytoalexins (phenol, alkaloids and tannins) in three varieties is due to their genetic differences. *P. fluorescens* showed its efficiency in inducing systemic resistance and increasing the plant's defense ability against sheath blight disease in rice caused by *R. solani*, and recorded an increase in the activity of the PAL, which led to an increase in the percentage of seed germination and the length of the root and shoot (Reshma *et al*., 2018). *Bacillus siamensis* strain LZ88 had significant antagonistic activity against various pathogenic fungi, especially against *Alternaria alternata*, which causes tobacco brown spots (Wang *et al*., 2022).

Conclusion

The results show that the addition of the biological control *T. harzianum* to *A. alternata* caused an increase in the activity of the enzyme PAL after 20 days from the start of the greenhouse experiment in the Spinal variety, compared with the enzyme activity in the treatment with *A. alternata* alone, while Netherland mulch Local Syria varieties showed different effects. The Netherland mulch variety recorded a lower phenolic content in all treatments, and there were more alkaloids and tannins than other varieties during the different periods. The difference in results may be due to the difference in the genetic structure of the studied varieties, which causes a difference in the response of the varieties to the formation of plant defenses, including phytoalexins.

ACKNOWLEDGEMENTS

The author appreciates the University of Mosul/College of Science for providing resources that aided in improving the quality of this work.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abbas, M.N., Rana, S.A., Shahid, M. , Rana, N., Mahmood-ul-Hassan, M. & Hussain, M. (2012). Chemical Evaluation of Weed seeds mixed with wheat grains at harvest. *J. Anim. Plant Sci.* 22(2), 283-288.
2. Al-Ameri, H. A. & Rammadan, N. A. (2022). Biological control mechanisms of *Trichoderma* species to induce resistance in Leguminous plants, Int. J. Adv. Multidisc. Res. Stud., 2(3),144-150.
3. Barnett, H.L. & Hunter, B.B. (2006). Illustrated genera of imperfect fungi. 4th Edition, The American Phytopatological Society, St. Paul Minnesota.
4. Basım, H. , Basım, E. , Baki, D. , Abdulai, M., Öztürk, N. & Balkic, R. (2018). Identification and characterization

of *Alternaria alternata* (Fr.) Keissler causing *Ceratonia* Blight disease of carob (*Ceratonia siliqua* L.) in Turkey, *European Journal of Plant Pathology*, v. 151, 73–86.

5. Beavdoin-Eagan, L. D. & Thorpe, T. A. (1985). Tyrosine and phenyl alanine ammonialysea activities during shoot initiation in tobacco callus, *Plant Physiol*., 78, 438 – 441.
6. Boham, B. A. & Kocipai-Abyazan, R. (1994).Flavonoids and condensed tannin from leaves of Hawaiian Vccinium vaticulatum and V. calycinium. *Pacific Sci.* 48, 458-463.
7. Cass, C.L., Antoine, P., Patrick, F.D., Yaseen, S., Nicholas, D.K., Steven, V.B., Yury, E.F.& Cliff & John, C.S. (2015). Effects of phenylalanine ammonia-lyase (PAL) knockdown on cell wall composition, biomass digestibility,and biotic and abiotic stress responses in Brachypodium. *Journal of Experimental Botany*. 66(14), 4317-4335.
8. Chen, Y., Fengjiao, L.i., Tian, L.u., Mingchao, H., Rufang, D. , Xueliu, L. , Wei, C., Pingzhi, W., Meiru, L., Huawu, J. & Guojiang, W. (2017). The phenylalanine ammonia-lyase gene LjPAL1 is involved 1 in plant defense responses to pathogens and plays diverse roles in Lotus japonicus-rhizobium symbioses. *Molecular Plant Microbe Interact*. 30(9), 739-753.
9. Crépon, K., Marget, P., Peyronnet, C., Carrouée, B., Arese, P.& Duc, G. (2010). Nutritional value of faba bean (Vicia faba L.) seeds for food and feed. *Field Crops Res.*, 115, 329–339. [CrossRef]
10. Ellis, M.B. (1971) Dematiaceous Hyphomycetes. Common wealth Mycological Institute, Kew, Surrey, England, 608.
11. Gveroska. B. & P. Jovancev, (2011). Some alternative biological ways for control of pathogenic fungus *Alternaria alternata* on tobacco. *Current Opinion in Biotechnology*, 22, 15-152.
12. Hahlborck, K. & Sheel, D. (1989). Physiology and molecular biology oh phenyl propanoid metsbolism, *Plant Mol. Biol.* , 40, 347 – 369.
13. Juroszek, P. A., Tiedemann, V.& Juroszek, P. (2011). Climate change will probably influence the occurrence, prevalence and sevariety of plant diseases. *Plant Patholog*., 60 (1), 100–112.
14. Kamble, P.U.,. Ramiah, M. & Patil. D. V. (2000). Efficacy of fungicides in controlling leaf spot disease of tomato caused by *Alternaria alternata* (F.) Kessiler. *J. Soils and Crops*, 10, 36-38.
15. Khazaei, H. & Vandenberg, A. (2020).Seed Mineral Composition and Protein Content of Faba Beans (*Vicia faba* L.) with Contrasting Tannin Contents, agronomy, 10, 511, p1-10, doi:10.3390/agronomy10040511.
16. Khazaei, H., Subedi, M., Nickerson, M., Martínez-Villaluenga, C., Frias, J.& Vandenberg, A. (2019). Seed protein of lentils: Current status, progress, and food applications. Foods, 8, 391. [CrossRef] [PubMed]
17. Kumar, P., Singh, S. K., Kumar, K., Kumar, A., Singh, A. & Kumar, A. (2020). Strategy on Management of *Alternaria* Leaf Spot of Broad Bean (Vicia faba L.) , *Int.J.Curr.Microbiol.App.Sci* (2020) Special Issue-11, 3181 -3189.
18. Lattanzio, V. (2013). Phenolic Compounds: Introduction. Natural Products., 1543-1580.
19. Małolepsza , U., Nawrocka, J. & Szczech, M. (2017). *Trichoderma virens* 106 inoculation stimulates defense enzyme activities and enhances phenolic levels in tomato plants leading to lowered Rhizoctonia solani infection. *Biocontrol Science Technology*. 27(2), 180-199.
20. Mhlongo, M. I., Piater, L.A., Madala, N.E., Labuschagne, N. & Dubery, I.A. (2018). The chemistry of plant–microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Frontiers in Plant Science*. 9 (112), 1-17.
21. Mssillou,I. , Bakour, M. , Slighoua, M. , Laaroussi, H. , Saghrouchni, H. , Amrati, F. , Lyoussi, B. & Derwich, E. (2022). Investigation on wound healing effect of Mediterranean medicinal plants and some related phenolic compounds: A review, *Journal of Ethnopharmacology*, V. 298, 15 November, 115663.
22. Reis, R. F., Almeida, T. F., Stuchi, E. S. & de Goes, A. (2007). Susceptibility of citrus species to *Alternaia alternata* the causal agent of the *Alternaria* brown spot. *Scientia Horticulturae*, 113 336-342:.
23. Reshma, P., Naik, M.K., Aiyaze, M., Niranjana, S.R., Chennappa, G., Shaikh, S.S. & Sayyed, R.Z. (2018). Induced systemic resistance by 2,4-diacetylphloroglucinol positive fluorescent Pseudomonas strains against rice sheath blight. *Indian Journal of Experimental Biology*. 56 (03), 207-212.
24. Rishi, K. M., Vidya, P. & Nehra, D.K. (2008). Study on phenolic and their Oxidative Enzyme in Capsicum annuum L. Infected with Geminivirus. *Asian J. Exp. Sci*. 22(3), 307-310.
25. Robinson, G.H.J., Balk, J., Domoney, C. (2019). Improving pulse crops as a source of protein, starch and micronutrients. *Nutr. Bull.*, 44, 202–215. [CrossRef] [PubMed]
26. Saydam, C., Copeu, M.& sezgin, E. (1973). Studies on the inoculation techniques of cotton wilt caused by *Verticillium dehliae* Kleb. Investigation on the laboratory inoculation techniques. *J. Turk. Phytopatho*., 2, 69 – 75.
27. Sen, S. (2017). Role of Phytoalexins in Plant-Microbe Interactions and Human Health. *International Journal of Scientific Research and Management*. 5(07), 6215-6225.
28. Sharma, C. K., Vishnoi, V. K., Dubey, R.C. & Maheshwari, D. K. (2018). A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen Macrophomina phaseolina in mung bean. Rhizosphere. 5:71-75.
29. Singh, R. & Chandrawat, K. S. (2017). Role of Phytoalexins in Plant Disease Resistance. *International Journal of Current Microbiology and Applied Sciences*, 6(1), 125-129.
30. Wang, D. , Li, Y. , Yuan, Y. , Chu, D. , Cao, J. , Sun, G. , Ai, Y. ,Cui, Z. , Zhang, Y. , Wang, F. & Wang, X. (2022).Identification of non-volatile and volatile organic compounds produced by *Bacillus siamensis* LZ88 and their antifungal activity against *Alternaria alternata, Biological Control*, V. 169, 104901.
31. Zhang, X. and Chang-Jun, L. (2015). Multifaceted Regulations of Gateway Enzyme Phenylalanine Ammonia-Lyase in the Biosynthesis of Phenylpropanoids. *Molecular Plant*. 8, 17-27.