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Original article

STUDY OF GENE EXPRESSION RESISTANCE IN OILSEED RAPE AGAINST PYTHIUM ROOT ROT CAUSED BY PYTHIUM SPP. FUNGI

J.T. Alkooranee

Abstract

Background. Pythium root rot (PRR), caused by multiple *Pythium* spp., is one of the most significant root diseases affecting Brassica crops. The use of chemical pesticides against PRR is an inappropriate due to the associated health and environmental risks to humans. Therefore, employing biotic and abiotic resistance elicitors presents a successful alternative for managing PRR in *Brassica napus*.

Materials and methods. *Bacillus subtilis*, *Chenopodium album* water extract, Salicylic acid, and Ayzox fungicide were used to analyze their effects on resistance genes involved in responses of *B. napus* to PRR. Three genes associated with plant defense, the JA signaling marker (*VSP2*), the ET signaling marker (*PR-4*), and the SA signaling marker (*PR-5*)- were examined for gene expression by quantitative real time-polymerase chain reaction (qRT-PCR).

Results. The results showed that plants inoculated with biotic and abiotic elicitors exhibited a reduction in the damping off, and the symptoms in these plants were less severe compared to those plants that were not inoculated with elicitors and were infected with PRR. The results of qPCR demonstrated that the expression levels of the *VSP2* and *PR-4* genes increased in plants infected with *Pythium* spp., while the expression levels of the *PR-5* gene increased in the leaves of plants treated with abiotic elicitors.

Conclusion. This study suggests that the biotic and abiotic elicitors used in the experiments are environmentally friendly agents and effective methods for protecting susceptible *B. napus* from *Pythium* spp.

Keywords: *Bacillus subtilis*; *Brassica napus*; *Chenopodium album* extract; *Pythium* spp.; Salicylic acid

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Introduction

Oilseed rape is a major crop with an increasing cultivation area across the world, but in Iraq, its cultivation remains limited and is widespread. However, the productivity of oilseed crop *Brassica napus* is still not sufficiently high, with a portion of the yield lost annually because of diseases.

Pythium spp. is one of the most diverse and widespread fungi of the Oomycets family worldwide, with more than 355 described species [1]. *Pythium* spp. are soilborne pathogens that cause adverse effects and severe economic losses in crops of the family Brassicaceae, leading to diseases such as damping-off, root and stem rots and leaf blights, which caused significant economic damage [2-4]. The protection of plants through the induction of systemic resistance is a promising and comprehensive approach to suppress many pathogens in the current context [5]. In Iraq, the management of Pythium root rot (PRR) diseases is based on prevention through the use of synthetic fungicides, with few studies exploring the use of biological and chemical agents in inducing systemic resistance. Biotic agents such as *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma viride* and *T. harzianum* have been tested for their ability to stimulate the systemic resistance in plants against various pathogens [5, 6].

Similarly, several studies indicate the role of plant extract in providing protection to plants against various pathogens. One study revealed that GC-MS analysis of *Chenopodium album* water extract showed the presence of methyl ester; 2 (3H)-furanone, 9-octadecenoic acid (Z), methyl ester; 9,12-octadecenoic acid (Z), dihydro-4,4-dimethyl; hexadecanoic acid, methyl ester; 1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester and 6-methylene bicyclo (3.2.0) hept-3-en-2-one has ability to control some plant disease fungi including *Rhizoctonia solani*, *Alternaria alternata*, *Sclerotinia sclerotium*, *Pythium aphanidermatum*, and *Fusarium solani* [7]. *C. album* extract significantly reduced the percentage of damping-off in bread wheat caused by *R. solani* and *F. solani* compared to the control treatment [8].

Biocontrol agents and plant extracts play an important role in protecting field crops by inducing systemic resistance against pathogens. Systemic resistance is classified into two types; induced systemic resistance (ISR) and systemic acquired resistance (SAR). ISR involves a promotion of plant defense activity to various pathogens through the application of Plant Growth Promoting Rhizobacteria (PGPR), while SAR is induced in plants through exposure to pathogenic microbes or treatment with abiotic elicitors [9].

ISR depends on ethylene (ET) and jasmonic acid (JA) pathways [10], while SAR is based on the salicylic acid (SA) pathway [11]. Salicylic acid and JA/

ET serve as regulatory hormones and are considered key signaling pathways for proteins and genes associated with plant resistance.

Defense and regulatory hormones have been used together to coordinate the host defense system. In earlier studies, certain genes associated with resistance have been observed in different plants in response to pathogen infections. Resistance genes, such as plant defenses in 1.2 (*PDF1.2*) and pathogenesis-related with proteins (*PR* families), including *PR-1, PR-2*, and *PR-5,* act directly or indirectly against pathogens. Genes of the pathogenesis-related proteins, like *PR-1, PR-2, TGA* transcription factor 5 and 6 are often used as markers for systemic acquired resistance (SAR) of the SA pathway. Whereas *PDF1.2,PR-3, PR-4*, the ethylene response factor 2 (*ERF2*) and allene oxide cyclase 3 (*AOC3*) genes, were used as markers of ISR in oilseed rape genotypes to *S. sclerotiorum* fungus [12, 13]. It is imperative to understand the relationship and interaction between the host and pathogens through the elicitor–receptor interaction. Acknowledging the relationships between the two is crucial for understanding the mechanisms of permanent resistance in plants and their evolution [14]. The aim of this study is to identify one or more hormonal signaling pathways involved in ISR and SAR by analyzing the responsive expression patterns of SA, JA and ET in *B. napus* treated with biotic or abiotic elicitors.

Materials and methods

Plant materials, pathogen and biotic

The genotype of *Brassica napus*, fungus *Pythium* spp. isolate and the abiotic elicitors SA , Ayzox fungicide (a new fungicide strobilurin & Hexaconazole 25% was tested as first time in lab) and the biotic elicitors *C. album* water extract and *B. subtilis* were prepared in the department of Field Crops, Faculty of Agriculture in Wasit University, Wasit city/ Iraq. The greenhouse and laboratory experiments were conducted in winter of 2023 in the Microbiology lab of the Field Crops department.

Pythium spp. was stored and cultured on an autoclaved Potato Dextrose Agar (PDA) medium in the dark at 20 to 25 ± 1°C. *B. subtilis* was cultivated for two days at 28 ± 2°C using pour-plate method on nutrient broth medium.

Invitro inhibition of mycelia length growth

The effect of chemical and biotic elicitors on the growth of Pythium spp. mycelia in dual-culture techniques was tested, where 100 ppm of Ayzox fungicide, 100 ppm of SA, 20 % of *C. album* water extract and 1×10^6 CFU/ml of *B. subtilis* as 5 ml of each elicitor agent were added separately to 100 ml of thawed P.D.A medium (40˚C ± 2˚C) that autoclaved for 30 min at 21˚C at 15 psi. Prior

to plating, then they mixed properly and then plates inoculated in the center separately with a 0.5 cm with mycelia plugs of *Pythium* spp., the plates for each combination of antagonist/ pathogen were replicated 3 times. As negative controls, 3 Petri dishes were inoculated only with a *Pythium* spp agar disc. After the fungal growth was completed in the control treatment plates, the radial growth and inhibition of mycelial pathogen were calculated according to the formula:

Percent inhibition = Pc - Pt / Pc x 100

Where PC = control plate, and Pt = treatment (radial growth of the pathogen towards the chemical and biotic elicitors).

Preparation of Pathogen Inoculum

This experiment was done by using the protocol which described by Alkooranee et al [13]. Erlenmeyer flasks were incubated at 25°C for 15 days with shaking daily by hand. By using the inoculum source, fungal suspension was adjusted to 1×10^5 fragments mL-1.

Examining elicitors agents on ISR-eliciting potential and disease impact

Experiments were designed under greenhouse conditions as follows: All seeds were t rinsed with sterile distilled water. To test the chemical and biotic elicitors they sterilized by 70% ethanol and 1% sodium hypochlorite solution for 1 min 5 min respectively. The sterilized seeds were soaked for 2 hours separately in elicitors suspensions as follows:

1- 30 seeds were submerged in distilled sterile water as a control treatment.

2- 15 seeds were submerged in 100 ppm of Ayzox fungicide.

3- 15 seeds were submerged in 100 ppm of Salicylic acid.

4- 15 seeds were submerged in 20 % of *Chenopodium album* extract.

5- 15 seeds were submerged in 1×10^6 CFU/ml of *B. subtilis*,

The treated seeds were cultivated as 5 seeds per pot (10-cm-diamter) containing peat moss and sand autoclaved at a ratio of 1:3. Seeds were grown at 22 °C with a cycle of 12 h/12 h light/dark and a light intensity of 150 µE/m2 /s.

Flasks of *Pythium* spp. inoculum were prepared in 2.3. paragraph, one day after applying the chemical and biological elicitors, the fungal pathogen inoculum was added to the seeds pots at a rate of 100 ml/pot and then mixed with the upper soil surface. Three duplicates of each treatment were used; three pots remained empty of the pathogen and elicitor agents however, as a control, only three pots inoculated with pathogen. The pots were irrigated twice a week.

Percentage germination (Germ %) evaluated after two weeks from planting:

No. of germinated seed /Total number of seeds cultured 100 ×

Thirty days after seed germination, shoot and root lengths (cm), fresh and dry shoot and root plants weight (g) of all seedlings were calculated on a sen-

sitive electronic weighing balance. The seedlings were placed in an electronic oven at 70 °C for 48 hours, and dry weights were recorded as well in this experiment.

Assay of Gene Expression

Twenty days after planting, two leaves were harvested from each treatment, and then frozen in liquid nitrogen. A whole RNA of *B. napus* leaves then extracted, quantitative real time-polymerase chain reaction (qRT-PCR) and first strand cDNA synthesis were performed by using Alkooranee et al [12] protocol. As shown in (Table 1), three primers of *B. napus* were used, consisting *VSP2*, *PR-4* and *PR-5* genes. On the other hand, the housekeeping gene (GAPDH) also used in this assay.

Table 1.

qRT-PCR primers used in this study

	Gene description	Pathway	Primer sequence (5′-3′)
PR-5	Pathogenesis-related protein 5	SA	ACCGCCACCATCTTCGTT GCCAGGGCAAATCTCGTT
VSP2	Vegetative storage protein 2	JA	TACCTCACTTCCGACCAG TTCTCAGTCCCGTATCCA
PR-4	Hevein-like protein	ET	GCCACCTACCATTACTACAAC TCCAAATCCAATCCTCCA
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase		CGCTTCCTTCAACATCATTCCCA TCAGATTCCTCCTTGATAGCCTT

Data analysis

By using GenStat software, statistical analysis was carried out using the least significant difference tests (LSD), means ($P < 0.05$) were compared between treatments [15].

Results and discussion

Identify the ability of invitro antagonists of biotic and abiotic elicitors

The results of the systemic resistance stimulating factors test showed a high ability to inhibit the growth of the pathogenic fungus in the dual medium culture. When using Ayzox fungicide and (SA), *C. album* water extract and *B. subtilis*, the growth rate of *Pythium* spp. fungus was reached 100%, 51.30%, 93.33% and 100% respectively (Figure 1).

The results of the present study are consistent with the previous studies that reported the fungicides, SA and biotic agents showing inhibition and antagonism against different soil-borne pathogens. Mihajlović et al [16] pointed out

that growth inhibition of *P. aphanidermatum* using chemicals propamocarb-hydrochloride (Previcur 607 SL), mefenoxam (Ridomil gold 480 SL) , mancozeb (Mankogal 80 WP) and azoxystrobin (Quadris) were 72.5%, 75.0% 57.5% and 77.5% respectively.

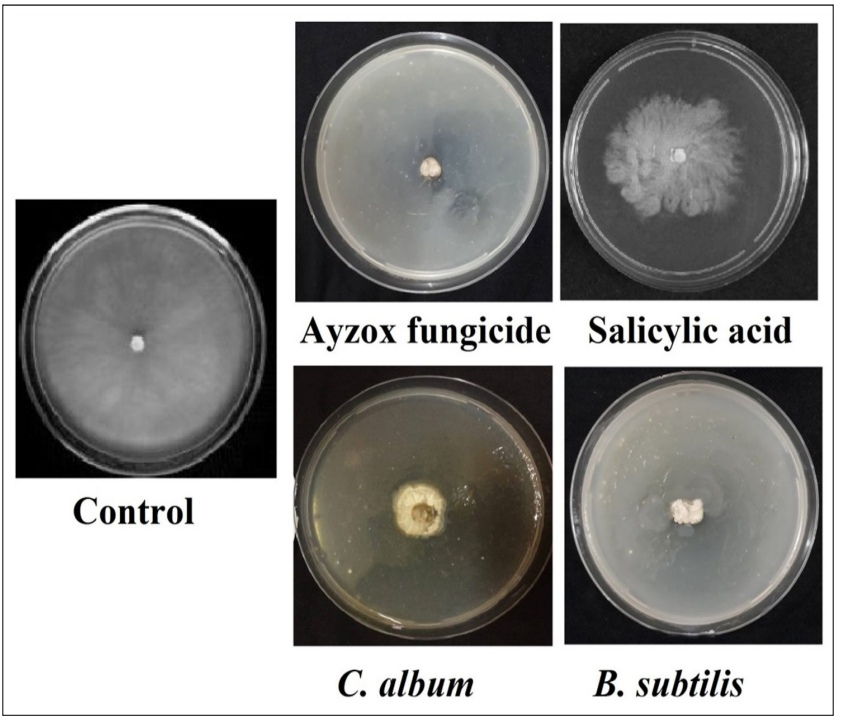

Figure 1. Invitro screening of elicitors agents against *Pythium* spp.

Ali et al [17] indicate that the ability of different root extract concentrations of *C. album* significantly reduced the growth of *Sclerotium rolfsii* fungus and biomass reached 15-58% compared to control. *B. subtilis* inhibited the mycelia growth of soil born fungi, such as *Pythium ultumum,* by uo to 86.6 % [18]. Salicylic acid and fungicides, vitavax 200 (V200), rizolex (Rz) and moncut (Mo) either individually or in combinations reduced linear growth (cm) of both *R. solani* and *F. solani* [19].

Bio-elicitors isolates are capable of producing volatile and non-volatile antibiotics, powerful plant degrading enzymes, and production of secondary me-

tabolism compounds such as ß-1,3 glucanase and chitinase enzymes. These enzymes lyse the mycelium of fungi by degrading the cell wall of the fungal pathogen [20].

Greenhouse Assays

The results of testing the ability of biological and chemical elicitors to stimulate resistance showed their effectiveness in inducing rapeseed resistance to PRR disease.

The percentage seed germination has been reached 73.33, 66.67, 80.00 and %100 in Ayzox, SA, *C. album* extract and *B. subtilis* and 33.33 % at pathogen treatment. The damping off test reached 0.00% for all elicitors' treatments, but it was 60% at pathogen treatment. However, the results identified that significant differences between vegetative growth parameters as high plant shoot fresh/dry weight and root fresh/dry weight of treated plants relative to comparison treatments (Table 2).

The *B. subtilis* treatment was the best in stimulating growth of the plant compared to the other treatments as the plants' height reached 14.90 cm, shoot fresh/dry weight 6.30 gm and 1.30 gm respectively, while root fresh/dry weight reached 1.54 gm and 0.163 gm (Table 2).

Systemic resistance is defined as the mechanism that protects plants by stimulating their defense against a wide range of pathogens, which is acquired after the presence of a suitable induction factor during infection. Biotic and abiotic elicitors in plants work together to improve plants health; ISR is important mechanisms by which selected PGPR in the rhizosphere system to strengthen the defense in the body of the plant against various pathogens [13]. Abiotic elicitors can be stimulate systemic resistance in plant through SA- signaling pathway and then turn-on gene resistance to pathogens attack [13; 21; 22].

Reports indicated that the plants growth has been effectively increased by the biotic elicitors. Alkooranee et al [6] found out that seed treated with *T. harzianum* increased the resistance and growth of plants. Secondary metabolism of PGPR act as resistance elicitor compound in variety of plants such as siderophore, pyoverdin, rhamnose (lipopolysaccharide), bacterial SA, fucose, flagellins and there enzymes that lead to ISR, including β-1,3-glucanase, chitinase, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, catalase, lipoxygenase, proteinase inhibitors, superoxide dismutase, and ascorbate peroxidase [23]. In one of the previous studies, the GC-MS analysis of the root extract of *C. alb*um revealed six compounds, may have an important role in inhibiting the growth of fungusmono (2-ethylhexyl) ester, 9-octadecenoic acid (Z)-, 1,2-benzenedicarboxylic acid, methyl ester, methyl ester, and 9-octadecenoic acid (Z)- [17].

Table 2.

Effect of elicitors agents on growth of oilseed rape infected by *pythium* spp. in greenhouse conditions

Treatments	Germ (%)	Damping off (%)	High plant (cm)	Shoot fresh weight (gm)	Root fresh weight (gm)	Shoot dry weight (gm)	Root dry weight (gm)
Control	66.67*	20.00	11.59	4.41	1.32	0.82	0.084
Pathogen	33.33	60.00	7.14	2.37	0.94	0.30	0.034
Ayzox	73.33	0.00	12.73	4.39	1.39	0.76	0.090
Salicylic acid	66.67	0.00	13.92	5.13	1.41	0.92	0.096
***C. album* extract**	80.00	0.00	13.48	5.45	1.30	1.12	0.121
B. subtilis	100	0.00	14.90	6.30	1.54	1.30	0.163
L.S.D	**14.15**	**18.84**	**3.11**	**1.62**	**0.22**	**0.14**	**0.013**

* Each value is a mean of three replications containing 5 seeds per plate.

Gene Expression

To determine the ability of *B. napus*, both treated and untreated with elicitors, to resist *Pythium* spp., three genes associated with resistant defense in *B. napus* leaves, including *VSP2, PR-4* and *PR-5,* have been detected by qRT-PCR technique after twenty days of planting. Hevein-like protein or the putative marker *PR-4* gene used as an indicator for the path of ET signal. While the *PR-5* (Pathogenesis-related protein 5) gene used as an indicator for the path of SA signal, however *VSP2* (Vegetative storage protein 2) used as an indicator for the path of JA signal.

Comparison of the Defense-Signaling Pathways in Healthy *B. Napus* Leaves or Infected By *Pythium* Spp.

Results demonstrated that, twenty days after planting, the high levels of expression of the *PR-5* gene increased by 3.14 -fold (from 1.21- to 3.80-fold) (Figure 2). *VSP2* is anti-pathogenic genes. For *VSP2* gene, the expression level increased in infected plants compared to non-infected ones, with a regulation of 1.28 fold (0.85 to 1.09 fold) (Figure 2).

The expression level of the *PR-4* gene showed that there were significant differences between infected and non-infected *B. napus*. They were up-regulated by 1.72-fold (from 1.12- to 1.93-fold) (Figure 2). It was clearly noticed that the defense response was low in infected plants, enabling the pathogenic fungus to grow rabidly and infect the plants. Low and delayed levels of expression of *PR-4* and *VSP2* genes were affected by the JA/ ET pathway. In fact, *Pythium* spp. saprophytic did not stimulate this pathway. The high level of *PR.5* gene expression may be related to SA pathway, which is induced by pathogenic micro-organisms

and, in turn, induces systemic acquired resistance or (SAR) in the host. Therefore, SAR is characterized by the induction of pathogenesis-related proteins.

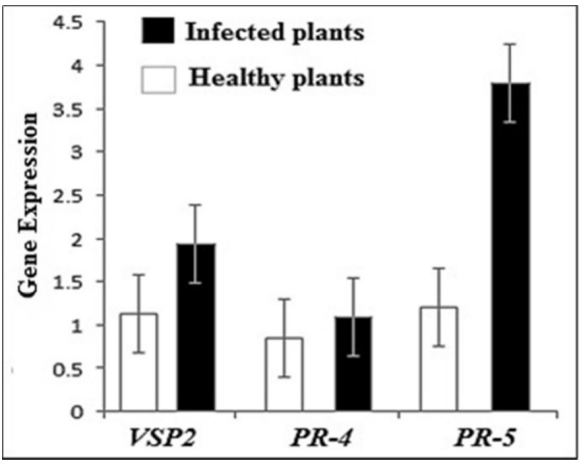

Figure 2. Expression levels of genes related to the resistance response after *B. napus* leaves were infected with *Pythium* spp, total RNA was extracted, cDNA was synthesized and the quantitative real-time polymerase chain reaction (qRT-PCR) was determined for three resistance genes *VSP2*, *PR-4* and *PR-5*

Several previous studies have identified that increased expression levels of resistance genes in oil seed rape genotypes infected with fungal pathogen compared to non-infected plants. The qRT-PCR results showed that the *PDF 1.2, AOC3* and *ERF2* genes (marker for the JA/ET signal pathway) expression levels increased in early plants infected by *S. sclerotiorum*, additionally to *PR-1* gene, but the expression levels decreased by *TGA5* and *TGA6* (marker for the SA signal pathway) [12]. The expression levels of resistance gene increased over time in oilseed rape genotypes inoculated with the fungus pathogen *Erysiph curciferarium,* causing powdery mildew disease compared to non-inoculated plants. The expression levels of *PR1* and *PR2* genes increased early in plants infected by *Sclerotinia sclerotiorum* pathogen, while the *PR-3* and *PDF1.2* genes, which serve as indicators for the path of the JA/ET signaling, increased later. This delay is because of the lifestyle of a pathogenic fungus as an obligate parasite [13].

Comparison of The Defense-Signaling Pathways in *B. Napus* Plants Treated And Non-Treated With Elicitors

In plants treated with Ayzox fungicide and infected by *Pythium* spp, the *PR-5* expression level, linked with SA pathway, was up-regulated by 19.06-fold

(3.80- to 72.45-fold) after twenty days of planting (Figure 3-A). However, the expression levels of the *VSP2* and *PR-4* genes were down-regulated in leaves treated with Ayzox fungicide, reaching 1.08-fold (1.93- to 3.20-fold) and 1.08-fold (1.09- to 5.10-fold), respectively (Figure 3-A).

In seeds treated with salicylic acid (SA) and then infected by pathogen after 24 hours, the *PR-5* gene expression level was up-regulated by 31.62.37-fold (Figure 3- B). Nevertheless, the expression levels of *VSP2* and *PR-4* genes showed no significant increase or a slight increase.

The results of gene expression levels in plants infected with *Pythium* spp. after 24 hours of treatment with C. album water extract showed that the expression of *VSP2* and *PR-4* genes was increased, being up-regulated by 26.85-fold (from 1.93- to 53.76-fold) and 36.51-fold (from 1.09- to 39.80-fold) respectively. However, the *PR-5* gene expression level in leaves treated with C. album water extract increased only slightly, reaching 2.52-fold (from 3.80- to 9.60-fold) (Figure 3-C).

In plants treated with *B. subtilis* and then infected by Pythium spp after 24 hours, the expression levels of *VSP2* and *PR-4* increased by 38.36-fold and 80.04-fold respectively. However the expression level of *PR-5* gene in plants was down-regulated by 1.08-fold (Figure 3-D).

Through the above results, it is noticed the success of biotic and abiotic elicitors to ISR in plant inoculated and infected by pathogen. The results showed that the abiotic elicitors depended on SA pathway, while biotic elicitors relied on JA and ET pathways. In plants treated with biotic factors, the genes associated with JA/ET pathway, *VSP2* and *PR-4,* were up-regulated, whereas the resistance gene related to SA-accumulation, *PR-5,* was down-regulated.

Many studies tested the ability of biotic elicitors to induce systemic resistance and activate resistance genes in different plants against many phytopathogens.

Chemicals that work as abiotic elicitors are capable of inducing systemic acquired resistance (SAR). It offers a number of features over current traditional techniques for disease control in plants. Even if only a portion of roots or shoots is sprayed during the seedling stage, the systemic acquired resistance (SAR) would be induced throughout the plant, providing long-term resistance.

The strobilurin fungicide, F500 (Pyraclostrobin), can induce systemic resistance in tobacco plants by acting downstream of SA in the SA signaling mechanism against tobacco mosaic virus (TMV) and *Pseudomonas syringae pv tabaci*, leading to accumulation of *PR-1* proteins in plants infected by TMV or in plants pre-treated with strobilurin fungicide than in the water-pretreated controls [24].

Salicylic acid plays an important role in activating systemic resistance in plants to different pathogens attack by stimulating some genes associated with

resistance. some studies conducted on this issue, including peanut against *Alternaria alternata* [25] and *Curcuma longa* (L.) against *Pythium aphanidermatuma* [26]. Salicylic acid is usually crucial in regulating acquired resistance (SAR) in hosts to defend against pathogens, resulting rapid SAR response.

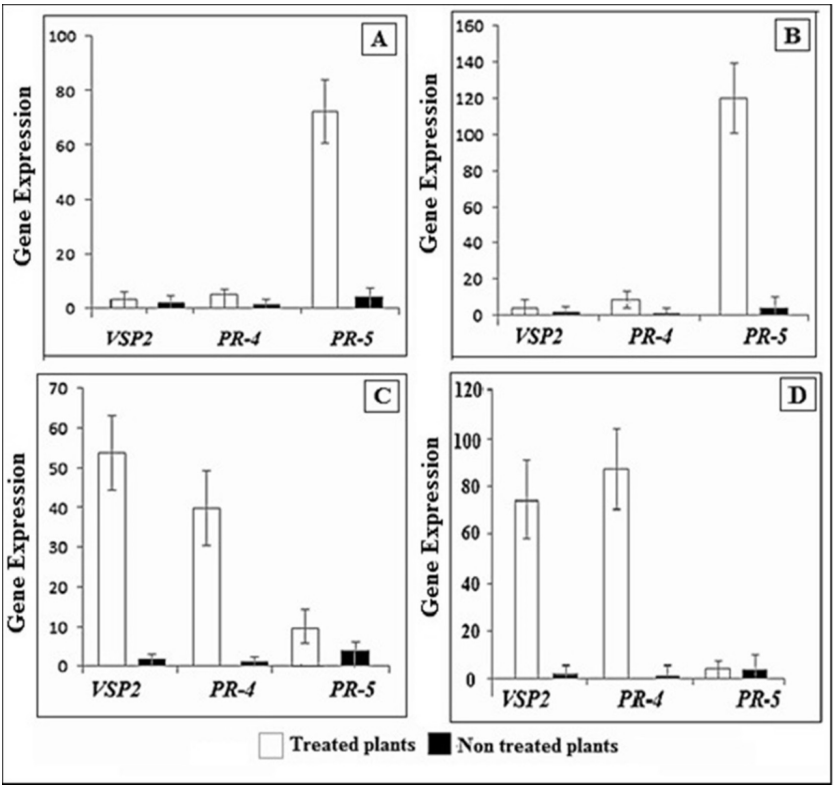

Figure 3. Expression levels of genes related to the resistance response in *B. napus* infected by *Pythium* spp. were treated with biotic and abiotic elicitors (A) Ayzox fungicide, (B) Salicylic acid, (C) *C. album* water extract, (D) *B. subtilis***.** total RNA was extracted, cDNA was synthesized and the quantitative real-time polymerase chain reaction (qRT-PCR) was determined for three resistance genes *VSP2, PR-4* and *PR-5***.**

The accumulation of various pathogenesis-related (PR) proteins suggests that the SA successfully induces systemic resistance in plants inoculated and infected with pathogen. The results indicate that the resistance is dependent on the SA pathway and independent of the JA/ET pathway. Specifically, the resistance gene related to SA

pathway, *PR-5,* was up-regulated in plants treated with SA, while the resistance genes associated with SA-accumulation, such as VSPE and *PR-4,* were down-regulated.

Salicylic acid operates through the SA signaling pathway in plants. This may be because SA acts as abiotic elicitor that activates SAR.

PR-1, PR-2 and *PR-5* genes, which are related to pathogen response and serve as markers for SAR dependent on SA, were examined in the shoots and roots of tomato plants infected by root-knot nematodes (RKNs). The results showed that the expression of these genes was up-regulated in plants pre-treated with SA, enhancing their resistance to the pathogen [27].

Cucurbit powdery mildew, caused by *Podosphaera fusca* fungus, was suppressed by *B. subtilis* UMAF6639, which was tested for its ability to induce systemic resistance in melon plants. This treatment led to the up-regulation of genes related to JA pathway, such as *LOX2* (lipoxygenase 2, as JA signal path indicators) and *PR-9* (peroxidase, as SA and JA signals path indicators). Howover, the *PR-1* gene, associated with SA pathway, was down-regulated. Before infection by *P. fusca*, plants treated with *B. cereus* UMAF8564 and *B. subtilis* UMAF6639 did not show a significant increase of *LOX2* expression compared to non-treated plants. After infection, the expression levels increased at 24 hpi in plants treated with UMAF8564 and at 48 hpi in those treated with UMAF6639 [28].

Conclusion

In Iraq, synthetic pesticides are widely used to control various field crop diseases. In the present study, the synthetic fungicide (Ayzox) was used to compare its effectiveness with some natural biological and chemical factors. The comparison was made by identifying the expression of resistance genes that rely on two different pathways (SA) and (JA/ETH). The present study showed that biological factors can be used as biocatalysts for systemic resistance, along with natural chemicals such as salicylic acid, which do not harm the environment or pose risks to human and animal health.

The study also demonstrated that the signaling pathways in plants depend on the type of elicitor. It was found that the resistance pathways in both systemic resistance types, ISA and SAR, are influenced by whether the elicitor is chemical or biological, leading to the stimulation of some genes associated with particular paths. The study also revealed that the type of pathogen plays a role in activating certain resistance genes.

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DATA ABOUT THE AUTHOR

Jawadayn Talib Alkooranee

Plant Department, College of Agriculture, Wasit University
Wasit, Iraq
jalkooranee@uowasit.edu.iq
ORCID: https://orcid.org/0000-0003-2997-4637
ResearchGate: https://www.researchgate.net/profile/Jawadayn-Alkooranee
Scopus Author ID: 56951124900

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