PLANT EXTRACTS INDUCING ENZYME ACTIVITY IN GRAINS AGAINST LOOSE SMUT DISEASE*

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This study investigated the role of endogenous Palestinian plant extracts in inducing wheat and barley resistance systems against loose smut disease with the aim to alternate the chemical pest control with natural fungicides. Twenty indigenous herbal plant extracts and essential oils were assessed for their biological and antifungal properties against *Ustilago tritici* and *Ustilago nuda*. Their potential role in inducing resistance pathways was studied on four different cultivars of wheat and barley. Two common enzyme indicators – guaiacol peroxidase (POX) and polyphenol oxidase (PPO) – are expressed in plants only after physical or chemical induction. The antifungal activity of the plant extracts was investigated *in vitro*. Totally 70 % of the plant extracts showed antifungal activity against *Ustilago tritici* and *Ustilago nuda*. Coridothyme extracts ranked first (61 %) in the fungal growth inhibition, followed by varthemia, salvia, ambrosia, artemisia, and lemon thyme. Some plant extracts significantly increased the POX and PPO effect compared to control for all the wheat and barley cultivars tested. The study revealed that oregano, clove or lavender and pomegranate, common yarrow or chamomile oil effectively induced the resistance indicator enzymes in wheat and barley.

wheat, barley, peroxidase, polyphenol-oxidase, *Ustilago tritici, Ustilago nuda*

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INTRODUCTION

Barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.), the most grown crop worldwide, are often attacked by many plant diseases, which causes severe economic losses (Wilcoxson, 1996). Barley loose smut *Ustilago nuda* Jens. Rostr. and wheat loose smut *Ustilago tritici* Pers. are very common in the West Bank, Palestine. There is a growing public concern about the effect of fungicide seed treatment on both animal and human health; on the other hand, recent records reported that loose smut had developed resistance to common fungicides used. Therefore, it is essential to search for alternative and safe methods toward sustainable agriculture production and food security (Tolman et al., 2004).

There has been a growing interest in exploiting indigenous plant extracts potential to control many crop diseases and insects. Herbal extracts and essential oils (EOs) display medicinal effects against many infectious diseases (Uma et al., 2017). Herbal crops and derived products have antispasmodic, antidepressant, anti-inflammatory, antioxidant, repellent properties and other effects, potentially supporting protection against targeted pests (Kazemi et al., 2010). In Palestine, herbs and home remedies are wildly used for their laxative, diuretic, analgesic, astringent, stimulant, and pain relief effects. They have been externally used to treat wounds, eczema, burns, also for sedation, digestive disorders, etc. Many studies reported on their essential role as plant resistance elicitors (Pinelo et al., 2004; Shabana et al.,

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Table 1. List of medicinal plants and commercially available oils used in the study

No.	Scientific name	English common name	Family	Part used
1	*Ajuga iva*	bugleweed	Lamiaceae	leaf
2	*Ambrosia artemisiifolia*	common ragweed	Asteraceae	leaf
3	*Ambrosia artemisiifolia*	common ragweed	Asteraceae	root
4	*Artemisia arborescens*	tree wormwood	Asteraceae	leaf
5	*Salvia fruticose*	Greek oregano	Lamiaceae	leaf
6	*Thymus*	green thyme	Lamiaceae	leaf
7	*Thymus citriodorus*	lemon thyme	Lamiaceae	leaf
8	*Coridothymu scapitatus*	coridothyme	Lamiaceae	leaf
9	*Achillea millefolium*	common yarrow	Asteraceae	leaf
10	*Varthemia persica*	foldy look	Asteraceae	leaf
11	*Matricaria recutita*	chamomile	Asteraceae	leaf
12	*Origanum vulgare*	oregano	Lamiaceae	leaf
13	*Rosmarinus officinalis*	rosemary	Lamiaceae	prepared oil
14	*Syzygium aromaticum*	clove	Myrtaceae	prepared oil
15	*Sinapis arvensis*	mustard	Brassicaceae	prepared oil
16	*Cinnamomum camphora*	camphor	Lauraceae	prepared oil
17	*Punica granatum*	pomegranate	Lythraceae	prepared oil
18	*Mentha spicata*	mint	Lamiaceae	prepared oil
19	*Salvia fruticose*	Greek oregano	Lamiaceae	prepared oil
20	*Lavandula spica*	lavender	Lamiaceae	prepared oil

2017). Plant pathogen defense level response measured by jasmonic acid and salicylic acid (SA) changes and the negative cross-talk response defense pathways have been extensively discussed (Cortes-Barco et al., 2010; Scott et al., 2017). Plant resistance elicitors could be chemical or biological-derived stimuli of crop systemic acquired resistance (SAR); such elicitors are activated by the SA pathway (Fu, Dong 2013). Dann et al. (1998) reported that using benzothiadiazole (BTH) or Actigard 50WG has been shown to enhance crop defense against *Pseudomonas syringae*. Walters et al. (2011) conducted several experiments on barley cultivars and compared crop growth rate after artificial inoculation with loose smut. They compared induced SAR activities in treated or untreated crops in combination with inhibitors of detoxification enzymes, antioxidants and multidrug resistance protein (MRP) transport.

Those studies aimed to clarify which pathogens might be negatively affected by plant SAR induced defenses (Walters et al., 2011). When plants were artificially inoculated with a pathogen, plant SAR-induced response was assessed for inhibitors of detoxification enzymes, antioxidants and MRP transport.

The current *in vitro* study aimed to investigate the potential antifungal activity of twenty Palestinian EOs against *Ustilago tritici* and *Ustilago nuda*, and to determine the enzymatic activity of wheat and barley cultivars after the fungal inoculation and EO treatment.

MATERIAL AND METHODS

Plant extracts

Herbal and medicinal plants and EOs (Table 1) were collected from several locations in West Bank, Palestina. Plant parts were dried at room temperature under controlled conditions in Kadoorie Agricultural Research Center (KARC) laboratories. The dried parts were ground to a fine powder and stored in air-tight glass bottles at 4 °C for further use. Methanol extracts were prepared as described by Onaran (2016). Totally 20 g of ground material of each plant was extracted with methanol at a ratio 1 : 3 (w/v) at 80 °C with agitation. Each homogenate was filtered through Macherey-Nagel filter paper. The subsequent aqueous solution was transferred to a rotator evaporator device (R201BC rotating rotary evaporator R205D-2L; *Shanghai Shen Sheng Technology* Co., Ltd., China) and adjusted at 65 °C. Concentrated extracts were then collected in dark air-tight bottles and stored at 4 °C.

Fungal isolation and maintenance

Wheat and barley plants infected with loose smut fungi were isolated and maintained following the methods described by Gilchrist-Saavedra (1997). Subculture was carried out on fresh potato dextrose agar (PDA) after 48 h and incubated at 35 ± 2 °C. Purified fungi isolates were maintained on PDA media every 2–3 weeks until the end of this study (Ravimannan et al., 2014). The fungi were identified according to colony morphology and colour and dikaryotic mycelium growth after a two-week incubation using an inverted microscope Optika XDS-2 Trinocular (AIPTEK International GmbH, Italy). Images were recorded with a HD 1080P digital camera (AIPTEK International GmbH).

Plant cultures maintenance

Wheat cultivars used in these experiments were Baladi (W1), Yellow Hiteyah (W2), Kahla (W3), and Nab al-Jamal (W4); barley cultivars were Baladi (B1), Lane 14/17 (B2), Rihane (B3), and ICARDA 176 (B4). Cultivars used in the experiments were propagated in the glasshouse in Kadoorie Agricultural Research Center (KARC) under 28 ± 2 °C and 60% relative humidity, fertilization was applied weekly with NPK (20 : 20 : 20) according to the supplier's recommendations.

Fungal inoculation

Each cultivar seedling was inoculated with the pathogen at the three-leaf stage by the injection method described by Schilling et al. (2014) and Quijano et al. (2016) (400 µl of 1.5×10^8 spores per 1 ml fungal suspension). All treated plants were maintained in glasshouse conditions, as mentioned above. The fungal inoculation experiment was conducted and maintained under glasshouse condition in 9 cm pots with 4–6 seeds with 3 replications for each treatment following a completely randomized block design. The treatments were: pathogen-inoculated plants (F), pathogen-free plants injured by needles (W), and control plants.

Assessment of plant enzymatic activities post fungal inoculation

Peroxidase (POX) and polyphenol oxidase (PPO) activities were measured spectrophotometrically. Wheat and barley cultivars were inoculated with pathogen based on the method described by Scott et al. (2017). At 48 h post-inoculation, all plant samples were frozen and kept at –80 °C until assay.

Assessment of plant extracts and EO treatment effects on host plants enzyme activity

The impact of twenty plant extracts and EOs (Table 1) on POX and PPO activities of four wheat and barley cultivars was assessed. Four seedlings of wheat or barley per pot were sprayed with one of the plant extracts or EOs. Treated crops were maintained in the glasshouse under the same conditions as described above. Plant samples were collected 48 h post-treatment and kept at –80 °C until assay.

For protein extraction, 3 g of leaves were homogenized with 1.25 µl of 0.1M potassium phosphate (K_3PO_4) buffer (pH 7, containing 7 % (w/v) polyvinylpyrrolidone). Totally 400 µl of Triton x-100 10% solution was added and vortexed for 10 s, then centrifuged for 15 min at 4 °C at 8 000 rpm (Hettich® MIKRO 200/200R centrifuge; Andreas Hettich GmbH & Co. KG, Germany). The supernatant was transferred to a new Eppendorf tube before measuring the enzyme activity. To determine the POX activity, 10 µl of the enzyme extract was added to a 2 ml disposable cuvette containing 1 ml of freshly prepared 5 mM guaiacol with 0.02 mM hydrogen peroxide (H_2O_2) dissolved in 0.1M K_3PO_4 buffer of pH 8. For the PPO assay, 10 µl of the enzyme extract was added to a 2 ml disposable cuvette containing 500 µl of freshly prepared 10 mM catechol dissolved in 0.1M K_3PO_4 buffer of pH 8. Changes in absorbance were measured at 470 nm for 30 s at room temperature using a spectrophotometer (DR6000 UV-VIS Spectro-photometer, Hach Lange, USA). The enzyme-specific activity for both the enzymes was reported as D in absorbance (optical density min^{-1} g^{-1} fresh tissue weight) (Jogaiah et al., 2020).

Essential oils bioactivity against *U. tritici* and *U. nuda*

The bio-efficacy of EOs (Table 1) against smut fungi was evaluated *in vitro*. A plate-hole diffusion technique (Scorzoni et al., 2007; Sales et al., 2016; Behbahani et al., 2017) was applied to screen the antifungal activity of 20 different EOs under aseptic conditions. Totally 70 µl of 1.5×10^6 spores per 1 ml fungal suspension counted under a hemocytometer (Ningbo Hinotek Technology Co., Ltd. Zhejiang, China)) (Gilchrist-Saavedra, 1997) were spread onto the surface of PDA media (60 mm Petri dish). Then, 5 mm of sterile disk filter papers soaked in extract (for around 10 min in aseptic condition) were placed over the middle of spread PDA media, with 3 replications per each EO treatment. Sterile filter papers soaked with distilled water were used as the first control, and a methanol-soaked filter paper was used as the second control. Petri dishes were labelled and incubated at 35 ± 2 °C (Rathod et al., 2015). The antifungal activity was evaluated by measuring the diameter of the inhibition zone (clear zone) of the fungal radial growth after 24 h and 72 h. The percentage inhibition of fungal growth was calculated according to Kumar, Tyagi (2013) and Aman, Rai (2015) using the formula:
Inhibition % = [(Diameter (mm) of control colony – Diameter (mm) of treated colony)/Diameter (mm) of control colony] × 100

Data analysis

All statistical analyses were performed using the SAS software (Statistical Analysis System, Version 2.0, 2008). Collected data were analyzed using ANOVA by the PROC GLM. The level of significance was determined using the Student-Newman-Keuls test. The means and standard deviations (SD) of 3 replicate readings were calculated on a 0.05 probability level.

RESULTS

Fungal inoculation

Results of wheat (Fig. 1) and barley (Fig. 2) seedlings inoculation at the three-leaf stage with the pathogens *U. tritici* and *U. nuda* are presented. The enzyme levels response in wheat and barley cultivars to the fungal injection and injury was significantly different compared with control. The PPO levels in W1 and W3 significantly differed from control (Fig. 1A), while in W2 and W4 they did not significantly differ from control. On the other hand, POX was significantly highly expressed in all wheat cultivars if compared to control (Fig. 1B). The PPO levels post fungal injection in barley cultivars enzymes were significantly less expressed in B1 and B2 than in control, while in B3 and B4 no significant differences were expressed compared with control (Fig. 2A). The POX levels post fungal injection were significantly lower in B1, B2 and B4 (Fig. 2B) compared with control.

The antifungal activity of plant extracts against *U. tritici* and *U. nuda*

The fungal growth inhibition percentage (FGIP) of *U. tritici* (Table 2) at 24 h post-treatment revealed that among all EOs used in the study, the coridothyme EO showed the highest antifungal effectiveness

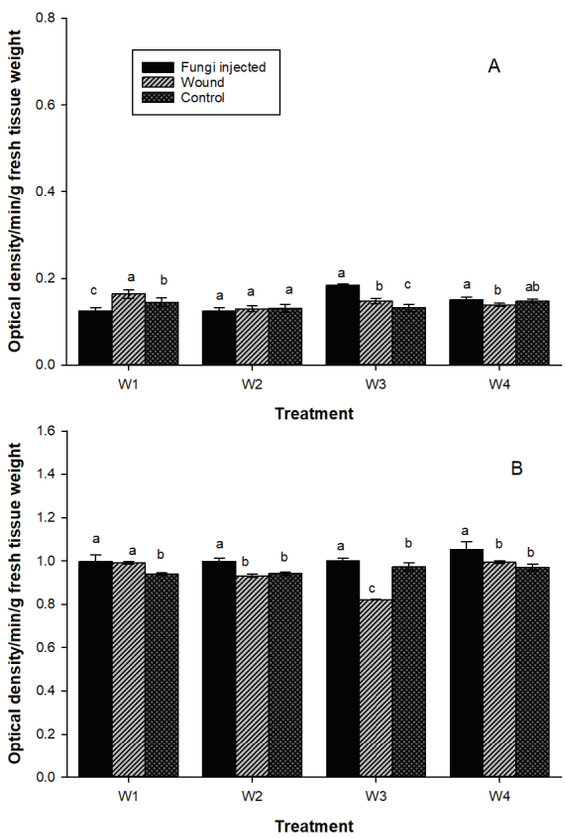

Figure 1. Changes in polyphenol oxidase (A) and peroxidase (B) in wheat seedlings injected with 400 µl of 1.5 × 108 fungal spores ml–1, wound injury by needles and control. Plant enzyme activities were measured as described in the text. Values are means of three replications ± SD 48 h post-treatment. Wheat cultivars were: Baladi (W1), Yellow Hiteyah (W2), Kahla (W3), and Nab-Al-Jamal (W4) *values followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

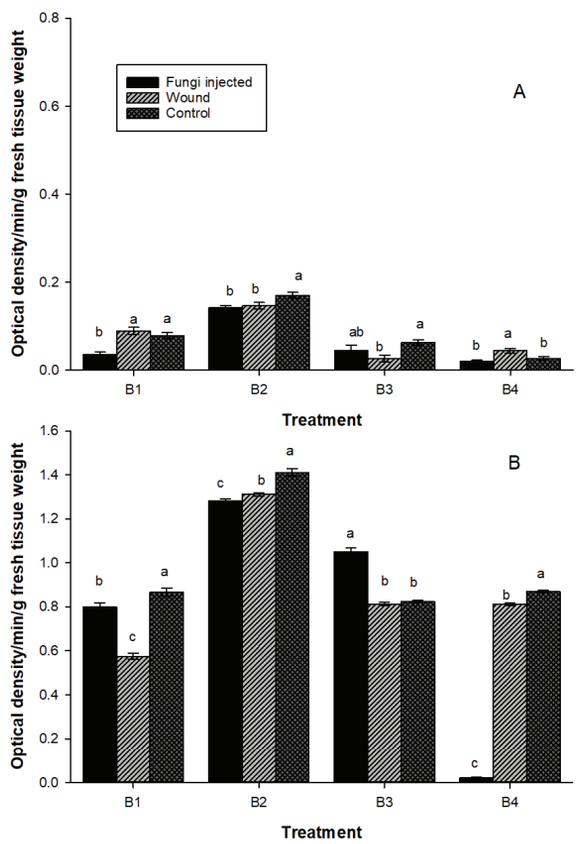

Figure 2. Changes in polyphenol oxidase (A) and peroxidase (B) in barley injected with 400 µl of 1.5 × 108 fungal spores ml–1, wound injury by needles and control. Plant enzyme activities were measured as described in the text. Values are means of three replications ± SD 48 h post-treatment. Barley cultivars were: Baladi (B1), Lane 14/17 (B2), Rihane (B3), and ICARDA 176 (B4) *values followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

Table 2. Efficacy of twenty essential oils and plant extracts on the fungal growth of Ustilago tritici and Ustilago nuda 24 h post-treatment *measurement of the percentage inhibition (values are means ± SD) of mycelium radial growth after 24 h. Means in a column followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

Plant extract	*Ustilago tritici*	*Ustilago nuda*
Ajuga	36.00 ± 0.00^{ed}	35.00 ± 10.00^{cd}
Ambrosia leaf	38.33 ± 2.08^{cd}	40.33 ± 4.04b^{cd}
Ambrosia root	32.33 ± 2.31^{ed}	40.33 ± 2.08^{bcd}
Artemisia	35.00 ± 3.46^{ed}	46.67 ± 0.58^{bc}
Salvia	49.00 ± 2.65^{b}	52.33 ± 6.66^{ab}
Green thyme	33.00 ± 5.29^{ed}	39.00 ± 3.61^{bcd}
Lemon thyme	39.00 ± 4.00^{cd}	42.00 ± 3.00^{bcd}
Coridothyme	59.00 ± 7.21^{a}	60.67 ± 8.08^{a}
Common yarrow	28.00 ± 4.24^{e}	30.00 ± 7.07^{d}
Varthemia	43.67 ± 4.04^{bc}	48.00 ± 3.61^{bc}
Chamomile	29.00 ± 1.73^{e}	35.33 ± 9.24^{cd}
Oregano	31.00 ± 0.00^{ed}	34.67 ± 9.50^{cd}
Rosemary oil	0^{f}	0^{e}
Clove oil	35.67 ± 3.06^{ed}	39.67 ± 2.52^{bcd}
Mustard oil	0^{f}	0^{e}
Camphor oil	0^{f}	0^{e}
Pomegranate oil	0^{f}	0^{e}
Mint oil	0^{f}	0^{e}
Salvia oil	31.67 ± 1.15^{ed}	38.33 ± 4.04^{bcd}
Lavender oil	0^{f}	0^{e}
Methanol	0^{f}	0^{e}
Water	0^{f}	0^{e}

(59.0 %), followed by salvia EO (49 %) and varthemia EO (43 %). The FGIP recorded for the remaining EOs was insignificant. The FGIP of *U. nuda* 24 h post-treatment revealed that the effect of coridothyme was the most significant compared with the other EOs and plant extracts (Table 2), showing a 60.7% inhibition, followed by salvia, varthemia and artemisia, (53 %, 48 %, 46.7 %, respectively). A non-significant antifungal activity, as demonstrated by the FGIP, was recorded for the rest of the EOs.

The effect of *U. tritici* injection on the wheat plant defense enzymes

The results for the PPO and POX specific activities induced by the fungal inoculation treatment are significantly variable; each wheat cultivar responded differently. For wheat cultivars, PPO activity readings generally had an increasing, similar, and decreasing effect. Meanwhile, POX activity readings generally had a slightly significant decreasing effect compared with water control at $P = 0.05$ post-treatment with the EOs mentioned in Table 1.

The PPO activity for wheat cultivars induced by fungal inoculation (Table 3) was significantly high post clove oil treatments in cultivar W1 compared with control, followed by rosemary oil and oregano, which had an increasing effect on the PPO level in W1 leaves (by 1.3 and 1.2 times, respectively). On the contrary, achille, coridothyme, salvia oil, artemisia, green thyme, lemon thyme, chamomile, varthemia, ambrosia root, mustard oil, salvia, ajuga, ambrosia leaf and camphor oil had a significant decreasing impact on the PPO activity with readings ranged from 0.104 to 0.031 optical density (OD) min^{-1} mg^{-1}. At the same time, pomegranate oil and mint oil had a similar impact on control. Methanol-treated plants exhibited a little significant decreasing effect on the W1 PPO activity. In W2 cultivar, the PPO activity was significantly increased in leaves treated with lavender oil (5.5 times over control), followed by salvia oil and pomegranate oil. The other EOs had a significant decreasing impact with values ranging between 0.1–0.04 OD min^{-1} mg^{-1}. Clove oil and rosemary oil had no significant effect compared to control. Methanol recorded a slightly high PPO activity compared with control. In W3 cultivar, the PPO activity reported a significant increment impact on leaves treated with lavender oil, clove oil, pomegranate oil and mint. There was no significant difference between salvia oil and salvia extract if compared with control. The rest of the EOs had a significant decreasing impact on the PPO level compared with control. The W4 PPO activity showed that lavender oil, clove oil and mustard oil-treated plants exhibited a significantly greater effect than control. Rosemary oil had an impact comparable with control. The remaining EOs had a significantly lower effect. Methanol treatment exhibited a significantly higher effect compared with control.

The POX activity results in post fungal inoculation of wheat cultivars are shown in Table 4. POX activity readings for W1 cultivar showed that oregano, salvia oil, clove oil and pomegranate oil had a significantly increasing impact compared with control. In contrast, other EOs exhibited a slight decrease in the POX value, except for rosemary oil, chamomile and varthemia that had a similar effect as control. In comparison, the methanol impact on POX activity was significantly increased compared with control. In W2 cultivar, the effects of all EOs on the POX activity showed a slightly significant decrease than their control values. Methanol readings pointed to its impact comparable with control (Table 4). The POX activity in W3 cultivar revealed that all EOs had a slightly significant decreasing effect than their control values. Compared to control, all EOs had a slightly significant decreasing effect on the POX activity in W4 cultivar (Table 4), while methanol readings pointed to a significant decreasing effect.

Table 3. Polyphenol oxidase (PPO) specific activity in four wheat cultivars leaves at 3rd foliar stage post fungal inoculation and twenty essential oils and plant extracts treatment. Results are means ± SD at P = 0.05. Enzyme activities were reported by Δ in absorbance (optical density min–1g–1 fresh tissue weight). Wheat cultivars were: Baladi (W1), Yellow Hiteyah (W2), Kahla (W3), and Nab-Al-Jamal (W4) *means in a column followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

Plant extract	W1	W2	W3	W4
Ajuga	0.05 ± 0.01^{i}	0.08 ± 0.00^{ij}	0.06 ± 0.00^{k}	0.08 ± 0.01^{g}
Ambrosia leaf	0.04 ± 0.00^{j}	0.04 ± 0.00^{m}	0.04 ± 0.00^{l}	0.04 ± 0.00^{j}
Ambrosia root	0.06 ± 0.01^{h}	0.06 ± 0.00^{kl}	0.05 ± 0.01^{k}	0.05 ± 0.00^{i}
Artemisia	0.08 ± 0.00^{g}	0.08 ± 0.00^{h}	0.10 ± 0.00^{fg}	0.10 ± 0.00^{d}
Salvia	0.06 ± 0.00^{hi}	0.06 ± 0.00^{k}	0.11 ± 0.01^{ef}	0.07 ± 0.01^{h}
Green thyme	0.07 ± 0.00^{g}	0.06 ± 0.01^{kl}	0.04 ± 0.00^{l}	0.04 ± 0.00^{j}
Lemon thyme	0.07 ± 0.00^{g}	0.09 ± 0.00^{g}	0.10 ± 0.00^{gh}	0.08 ± 0.01^{fg}
Coridothyme	0.10 ± 0.00^{e}	0.07 ± 0.00^{j}	0.05 ± 0.00^{k}	0.05 ± 0.00^{i}
Common yarrow	0.10 ± 0.00^{de}	0.07 ± 0.00^{ij}	0.09 ± 0.00^{h}	0.05 ± 0.00^{i}
Varthemia	0.07 ± 0.00^{g}	0.08 ± 0.00^{h}	0.07 ± 0.00^{j}	0.07 ± 0.01^{h}
Chamomile	0.07 ± 0.00^{g}	0.10 ± 0.00^{f}	0.10 ± 0.00^{gh}	0.07 ± 0.00^{h}
Oregano	0.15 ± 0.00^{b}	0.08 ± 0.00^{i}	0.08 ± 0.00^{i}	0.08 ± 0.00^{fg}
Rosemary oil	0.15 ± 0.00^{b}	0.11 ± 0.00^{e}	0.07 ± 0.01^{j}	0.11 ± 0.00^{c}
Clove oil	0.18 ± 0.00^{a}	0.11 ± 0.00^{e}	0.21 ± 0.00^{b}	0.26 ± 0.01^{a}
Mustard oil	0.06 ± 0.00^{hi}	0.09 ± 0.00^{g}	0.07 ± 0.00^{j}	0.12 ± 0.00 ^{b}
Camphor oil	0.03 ± 0.00^{k}	0.05 ± 0.00^{l}	0.03 ± 0.00^{m}	0.03 ± 0.00^{k}
Pomegranate oil	0.12 ± 0.00^{c}	0.23 ± 0.00^{c}	0.19 ± 0.00^{c}	0.04 ± 0.00^{j}
Mint oil	0.12 ± 0.00^{c}	0.10 ± 0.00^{g}	0.13 ± 0.00^{d}	0.09 ± 0.01^{de}
Salvia oil	0.09 ± 0.00^{f}	0.25 ± 0.00^{b}	0.11 ± 0.00^{ef}	0.09 ± 0.00^{ef}
Lavender oil	0.12 ± 0.00^{c}	0.61 ± 0.00^{a}	0.44 ± 0.00^{a}	0.26 ± 0.00^{a}
Methanol	0.11 ± 0.00^{d}	0.13 ± 0.00^{d}	0.11 ± 0.00^{e}	0.11 ± 0.00^{c}
Water	0.12 ± 0.00^{c}	0.11 ± 0.00^{e}	0.11 ± 0.00^{e}	0.11 ± 0.00^{c}

Table 4. Guaiacol peroxidase (POX) specific activity in four wheat cultivars leaves at 3rd foliar stage post fungal inoculation and twenty essential oils and plant extracts treatment. Results are means ± SD at P = 0.05. Enzyme activities were reported by Δ in absorbance (optical density min–1g–1 fresh tissue weight). Wheat cultivars were: Baladi (W1), Yellow Hiteyah (W2), Kahla (W3), and Nab-Al-Jamal (W4) *means in a column followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

Plant extract	W1	W2	W3	W4
Ajuga	1.12 ± 0.01^{i}	1.23 ± 0.00^{ef}	1.15 ± 0.00^{j}	1.15 ± 0.01^{g}
Ambrosia leaf	1.16 ± 0.00^{g}	1.23 ± 0.00^{d}	1.15 ± 0.00^{j}	0.85 ± 0.00^{m}
Ambrosia root	1.17 ± 0.00^{f}	1.14 ± 0.00^{j}	1.15 ± 0.00^{j}	0.99 ± 0.01^{l}
Artemisia	1.14 ± 0.00^{h}	1.21 ± 0.01^{h}	1.19 ± 0.01^{f}	1.04 ± 0.00^{k}
Salvia	1.04 ± 0.00^{m}	0.94 ± 0.01^{n}	1.16 ± 0.00^{i}	1.17 ± 0.00^{f}
Green thyme	0.98 ± 0.01^{o}	1.20 ± 0.00^{i}	1.09 ± 0.00^{l}	1.12 ± 0.00^{h}
Lemon thyme	1.11 ± 0.01^{j}	1.20 ± 0.00^{i}	1.24 ± 0.00^{b}	1.13 ± 0.01^{h}
Coridothyme	1.12 ± 0.00^{i}	1.14 ± 0.00^{j}	1.09 ± 0.01^{m}	1.15 ± 0.01^{g}
Common yarrow	1.09 ± 0.01^{k}	1.22 ± 0.00^{fgh}	1.18 ± 0.00^{g}	1.25 ± 0.01^{b}
Varthemia	1.19 ± 0.01^{e}	1.13 ± 0.00 ^{j}	1.17 ± 0.00^{h}	1.15 ± 0.00^{g}
Chamomile	1.19 ± 0.00^{e}	1.10 ± 0.00^{l}	1.13 ± 0.01^{k}	1.10 ± 0.00^{i}
Oregano	1.25 ± 0.01^{b}	1.22 ± 0.00^{gh}	1.22 ± 0.00^{c}	1.21 ± 0.01^{d}
Rosemary oil	1.21 ± 0.00^{d}	1.24 ± 0.01^{d}	1.21 ± 0.00^{d}	1.15 ± 0.00^{g}
Clove oil	1.23 ± 0.00^{c}	1.22 ± 0.00^{fgh}	1.20 ± 0.00^{ef}	1.22 ± 0.00^{c}
Mustard oil	1.19 ± 0.00^{e}	1.23 ± 0.00^{de}	1.17 ± 0.00^{h}	1.15 ± 0.01^{g}
Camphor oil	1.02 ± 0.00^{n}	1.22 ± 0.00^{fgh}	1.15 ± 0.01^{j}	1.20 ± 0.00^{e}
Pomegranate oil	1.21 ± 0.01^{d}	1.06 ± 0.00^{m}	1.17 ± 0.01^{h}	1.06 ± 0.01^{j}
Mint oil	1.07 ± 0.01^{l}	1.11 ± 0.00^{k}	1.15 ± 0.00^{ij}	1.15 ± 0.00^{g}
Salvia oil	1.23 ± 0.01^{c}	1.22 ± 0.00^{fg}	1.20 ± 0.00^{de}	1.21 ± 0.00^{d}
Lavender oil	1.19 ± 0.00^{e}	1.28 ± 0.00^{c}	1.21 ± 0.00^{d}	1.20 ± 0.00^{de}
Methanol	1.27 ± 0.00^{a}	1.29 ± 0.00^{b}	1.22 ± 0.00^{c}	1.20 ± 0.01^{de}
Water	1.20 ± 0.00^{de}	1.41 ± 0.00^{a}	1.29 ± 0.00^{a}	1.27 ± 0.01^{a}

The effect of *U. nuda* injection on barley plants defense enzymes

In barley cultivars, the PPO levels post fungal inoculation (Table 5) were significantly lower in cultivars B1 and B2, while there was no significant impact in B3 and B4 cultivars if compared to control. In B1 cultivar, treatment with common yarrow, varthemia, rosemary oil, chamomile, salvia, coridothyme, pomegranate oil, artemisia, green thyme, ajuga, lavender oil, clove oil, oregano, ambrosia leaf, lemon thyme, salvia oil, mustard oil and camphor oil had a significantly increasing impact on the PPO activity compared with control, while ambrosia root and mint oil had no impact compared to control. Methanol-induced PPO activity was slightly higher than that of control. The PPO activity results in B3, where plants were treated with artemisia extracts, recorded the highest value, followed by varthemia, common yarrow, chamomile, green thyme, coridothyme, clove oil, camphor oil, ajuga, lemon thyme, rosemary oil, oregano, lavender oil, mint oil, ambrosia root, mustard oil, salvia, ambrosia leaf and pomegranate oil with readings ranged from 0.648 to 0.078 OD min^{-1} mg^{-1}. Salvia oil treatment showed no significant difference from control. The methanol-induced PPO activity was the same like in control. The PPO activity results for B4 cultivar treated plants are shown in Table 5. Data indicate that oregano and common yarrow increased the PPO values over the control value, followed by chamomile, coridothyme, varthemia, clove oil, rosemary oil, ambrosia root, ajuga, camphor oil, ambrosia leaf, pomegranate oil, lavender oil, mustard oil, mint oil, lemonthyme and green thyme, which increased PPO values significantly over their control value. In contrast, artemisia displayed a decreased PPO activity compared with control. In comparison, salvia and salvia oil had the same effect as control. The methanol-induced PPO activity level was significantly higher than that of control. The POX level post fungal inoculation (Table 6) was significantly lower for B1, B2 and B4 cultivars, compared to their control values. In B3 cultivar, the POX level was significantly higher post fungal inoculation compared with control reading. The POX activity after treating the B3 cultivar plants with pomegranate oil, mint oil and lavender oil was significantly higher than in control. However, the effect of salvia oil was slightly significantly decreased. The rest of EOs greatly decreased the POX activity values compared with control. Readings related to the methanol treatment impact on the POX-specific activity showed significantly lower values than control. In B2 cultivar, the PPO activity results show that pomegranate oil treatment attained by 4.9 times higher readings than control. Varthemia, common yarrow, chamomile, oregano, green thyme, artemisia, ajuga, lemon thyme, ambrosia leaf, salvia, camphor oil, and mint oil followed, while coridothyme, lavender oil, salvia oil and ambrosia root affected the PPO activity similarly as control. Mustard oil, clove oil, and rosemary oil decreased the PPO levels compared to control levels. Methanol treated B2 cultivar leaves recorded significantly lower PPO levels than control. The POX specific activity for B2 cultivar evoked by all EOs except pomegranate oil, mint oil, lavender oil and salvia oil significantly decreased compared with control. Pomegranate oil recorded a slightly more significant value than control. The POX specific activity in B3 cultivar, after treating the plants with all EOs except pomegranate oil, mint oil, lavender oil and salvia oil, significantly decreased compared with control. Lavender oil recorded a slightly significantly higher value than control, while the impact of salvia oil, mint oil and pomegranate oil on the POX activity was slightly decreasing. Readings related to the methanol treatment impact on the POX specific activity showed a significantly lower value for B3 cultivar than control.

On the other hand, the POX specific activity for B4 cultivar stimulated by all EOs except pomegranate oil, mint oil, lavender oil and salvia oil, significantly decreased compared with control. The B4 cultivar plants treated with mint oil, salvia oil, pomegranate oil and lavender oil displayed a slightly higher significant POX activity than their controls. Readings related to the methanol treatment impact on their POX specific activity showed similar results if compared with control.

DISCUSSION

The study revealed that 70 % of the medicinal EOs used against *U. tritici* and *U. nuda* fungi *in vitro* had some antifungal activity potential. The inhibition percentage of both plant pathogens ranged between 33–60 %. Our results are in line with those obtained in several other studies.

Leaf extracts of poisonous phanergamic, *Nerium odorum*, senna and Indian mallow are used in controlling wheat rust fungi; different plant extracts of e.g. *Azardiachta indica* (Ayoub, Niazi, 2001; Dey et al., 2013), *Artemisia annua* (Pouresmaeil et al., 2020), *Eucalyptus globulus*, *Ocimum sanctum* and *Rheum emodi* (Joseph et al., 2008; Caetano et al., 2020) were tested to control wilt pathogen in brinjal (Rahber-Bhatti, 1988). On the other hand, plants generally can develop resistance after a pathogen invasion, insect infestation, rhizomicrobes (biotech) or chemical/physical agents (abiotech) treatments (Pieterse et al., 2014). Six defense signaling pathways have reportedly been involved in different regulators depending on the type of infection or related situations (Ton et al., 2002). Host plants consume their energy and resources to balance their growth and defending mechanisms, over cross-talking between plant hormones and resources, protecting their system from abiotech and biotech stress sacrificing

Table 5. PPO specific activity in four barley cultivars leaves at 3rd foliar stage post fungal inoculation and twenty essential oils and plant extracts treatment. Results are means ± SD at P = 0.05. Enzyme activities were reported by Δ in absorbance (optical density min–1g–1 fresh tissue weight). Barley cultivars were Baladi (B1), Lane 14/17 (B2), Rihane (B3), and ICARDA 176 (B4) *means in a column followed by the same letter are not significantly different (Student-Newman-Keuls test, P > 0.05)

Plant extract	B1	B2	B3	B4
Ajuga	0.22 ± 0.01^{h}	0.22 ± 0.00^{f}	0.23 ± 0.02^{g}	0.23 ± 0.01^{f}
Ambrosia leaf	0.13 ± 0.01^{j}	0.20 ± 0.01^{g}	0.12 ± 0.01^{jk}	0.16 ± 0.01^{gh}
Ambrosia root	0.08 ± 0.00^{mn}	0.13 ± 0.01^{i}	0.16 ± 0.01^{i}	0.23 ± 0.01^{f}
Artemisia	0.28 ± 0.01^{f}	0.28 ± 0.02^{e}	0.76 ± 0.01^{a}	0.02 ± 0.00^{m}
Salvia	0.35 ± 0.00^{d}	0.20 ± 0.00^{g}	0.12 ± 0.00^{jk}	0.09 ± 0.01^{k}
Green thyme	0.24 ± 0.01^{g}	0.35 ± 0.01^{d}	0.38 ± 0.01^{e}	0.12 ± 0.00^{j}
Lemon thyme	0.11 ± 0.00^{k}	0.22 ± 0.01^{f}	0.23 ± 0.02^{g}	0.13 ± 0.01^{j}
Coridothyme	0.32 ± 0.01^{e}	0.14 ± 0.00^{i}	0.35 ± 0.00^{f}	0.32 ± 0.01^{d}
Common yarrow	0.47 ± 0.01^{a}	0.39 ± 0.01^{c}	0.59 ± 0.01^{c}	0.54 ± 0.01^{b}
Varthemia	0.41 ± 0.00^{b}	0.56 ± 0.01^{b}	0.65 ± 0.01^{b}	0.28 ± 0.01^{e}
Chamomile	0.39 ± 0.01^{c}	0.37 ± 0.01^{d}	0.51 ± 0.03^{d}	0.34 ± 0.01^{c}
Oregano	0.20 ± 0.01^{i}	0.36 ± 0.00^{d}	0.18 ± 0.01^{hi}	0.56 ± 0.01^{a}
Rosemary oil	0.41 ± 0.01^{b}	0.07 ± 0.00^{k}	0.20 ± 0.02^{h}	0.26 ± 0.01^{e}
Clove oil	0.20 ± 0.01^{i}	0.10 ± 0.00^{j}	0.25 ± 0.00^{g}	0.27 ± 0.01^{e}
Mustard oil	0.11 ± .01^{kl}	0.11 ± 0.00^{j}	0.14 ± 0.01^{j}	0.14 ± 0.00^{hi}
Camphor oil	0.09 ± 0.01^{lm}	0.16 ± 0.00^{h}	0.25 ± 0.01^{g}	0.17 ± 0.01^{g}
Pomegranate oil	0.31 ± 0.01^{e}	0.66 ± 0.01^{a}	0.11 ± 0.01^{k}	0.15 ± 0.01^{hi}
Mint oil	0.06 ± 0.01^{o}	0.16 ± 0.00^{h}	0.17 ± 0.01^{i}	0.14 ± 0.00^{ij}
Salvia oil	0.11 ± 0.00^{kl}	0.13 ± 0.01^{i}	0.08 ± 0.01^{l}	0.07 ± 0.01^{l}
Lavender oil	0.21 ± 0.00^{hi}	0.13 ± 0.01^{i}	0.17 ± 0.01^{i}	0.15 ± 0.01^{hi}
Methanol	0.10 ± 0.00^{kl}	0.11 ± 0.01^{j}	0.06 ± 0.01^{l}	0.10 ± 0.00^{k}
Water	0.07 ± 0.01^{no}	0.14 ± 0.01^{i}	0.07 ± 0.01^{l}	0.23 ± 0.01^{l}

Table 6. POX specific activity in four barley cultivars leaves at 3rd foliar stage post fungal inoculation and twenty essential oils and plant extracts treatment. Results are means ± SD at P = 0.05. Enzyme activities were reported by Δ in absorbance (optical density min–1g–1 fresh tissue weight). Barley cultivars were Baladi (B1), Lane 14/17 (B2), Rihane (B3), and ICARDA 176 (B4) *means in a column followed by the same superscript are not significantly different (Student-Newman-Keuls test, P > 0.05)

Plant extract	B1	B2	B3	B4
Ajuga	0.02 ± 0.00^{kl}	0.04 ± 0.01^{h}	0.07 ± 0.00^{kl}	0.04 ± 0.00^{m}
Ambrosia leaf	0.05 ± 0.00^{ijk}	0.00 ± 0.00^{i}	0.03 ± 0.00^{po}	0.05 ± 0.00^{kl}
Ambrosia root	0.02 ± 0.00^{l}	0.07 ± 0.00^{gh}	0.08 ± 0.00^{j}	0.06 ± 0.00^{jk}
Artemisia	0.11 ± 0.00^{h}	0.08 ± 0.01^{ef}	0.10 ± 0.00^{h}	0.04 ± 0.00^{m}
Salvia	0.06 ± .00^{ij}	0.08 ± 0.00^{ef}	0.09 ± 0.01^{i}	0.08 ± 0.00^{i}
Green thyme	0.05 ± 0.01^{ijk}	0.04 ± 0.01^{gh}	0.05 ± 0.00^{n}	0.05 ± 0.00^{l}
Lemon thyme	0.06 ± 0.00^{ij}	0.06 ± 0.01^{fgh}	0.06 ± 0.00^{lmn}	0.05 ± 0.00^{kl}
Coridothyme	0.07 ± 0.01^{ij}	0.05 ± 0.01^{efg}	0.08 ± 0.00^{j}	0.05 ± 0.00^{jkl}
Common yarrow	0.07 ± 0.00^{i}	0.05 ± 0.00^{egh}	0.05 ± .01^{mn}	0.08 ± 0.00^{i}
Varthemia	0.07 ± 0.00^{i}	0.07 ± 0.00^{fg}	0.06 ± 0.00^{lmn}	0.03 ± 0.00^{n}
Chamomile	0.11 ± 0.00^{h}	0.07 ± 0.01^{fg}	0.07 ± 0.01^{jk}	0.11 ± 0.00^{g}
Oregano	0.06 ± 0.01^{ij}	0.06 ± 0.01^{fgh}	0.12 ± 0.00^{g}	0.09 ± 0.00^{h}
Rosemary oil	0.31 ± 0.01^{g}	0.03 ± 0.00^{h}	0.07 ± 0.00^{lmn}	0.12 ± 0.01^{g}
Clove oil	0.11 ± 0.01^{h}	0.05 ± 0.00^{fgh}	0.07 ± 0.01^{lmn}	0.06 ± 0.00^{j}
Mustard oil	0.04 ± 0.00^{kl}	0.06 ± 0.01^{fgh}	0.03 ± 0.00^{p}	0.03 ± 0.00^{m}
Camphor oil	0.04 ± 0.00^{ijkl}	0.11 ± 0.00^{e}	0.04 ± 0.01^{o}	0.04 ± 0.00^{m}
Pomegranate oil	1.25 ± 0.03^{a}	1.42 ± 0.05^{a}	1.00 ± 0.00^{f}	1.10 ± 0.01^{c}
Mint oil	1.19 ± 0.02^{b}	1.29 ± 0.01^{c}	1.19 ± 0.00^{d}	1.20 ± 0.00^{a}
Salvia oil	0.99 ± 0.02^{e}	1.26 ± 0.01^{d}	1.28 ± 0.00^{c}	1.19 ± 0.00^{b}
Lavender oil	1.12 ± 0.03^{c}	1.25 ± 0.00^{d}	1.33 ± 0.00^{a}	1.08 ± 0.01^{d}
Methanol	0.93 ± 0.00^{f}	1.24 ± 0.00^{d}	1.04 ± 0.00^{e}	0.87 ± 0.00^{f}
Water	1.04 ± 0.00^{d}	1.33 ± 0.00^{b}	1.32 ± 0.00^{b}	1.07 ± 0.00^{e}

their normal growth and development (Fu, Dong, 2013). The effects of developed plant resistance were correlated with those induced by several antimicrobial compounds, e.g. reducing oxide enzymes like PPO, POX and others (Chittoor et al., 1999). However, the exact function of POX in inducing the resistance mechanisms in plants has still been ambiguous (Datta, Muthukrishnan, 1999). Yet, several studies reported on the potential function referring to POX and PPO (Aditya, Hegde, 2020). Several researches described changes in the POX activity in wheat after stem rust infection (Flott et al., 1989) and in barley after powdery mildew infection (Kerby, Somerville, 1989) in response to plant-induced resistance. Those indicator enzymes have also been found in many other plants post a pathogenic attack, e.g. in cabbage infected with *Fusarium oxysporum*, onion infected with *Botrytis* sp., sunflower infected with *Sclerotinia sclerotiorum,* soybean infected with *Phytophthorameg asperma* and bean infected with *Rhizoctonia* sp. (Datta, Muthukrishnan, 1999).

Moreover, when studying the elicitors–plant interaction, the pathogen-related enzyme activation after chemical applications mimicking a disease infection or injury responses have frequently been described (Fu, Dong, 2013; Pieterse et al., 2014). Similar results have been reported in many crops such as lettuce, tomato, apple, cabbage, and pea after applying β-aminobutyric acid (BABA) (van der Wolf et al., 2012). An increased gene expression of POX and PPO in mango plants (Lin et al., 2011) and muskmelon (Zhang et al., 2011) has been registered after acibenzolar-s-methyl (ASM) application. On the other hand, some studies reported on a decreased POX activity in *Brassica napus* after SA and nitric oxide elicitors application (Kazemi et al., 2010), while treating wheat heads with SA elicitors did not induce the gene expression of POX (Mohammadi, Kazemi, 2002).

Changes in plant PPO and POX levels mentioned in this study could be associated with different factors, such as plant growth stage, plant genotype, type of elicitor and their action. But mostly the induced resistance expression is plant genotype-dependent (Tucci et al., 2011). The treatment of spring barley cultivars with a combination of BABA, ASM and *cis*-jasmone resulted in a significant variability in the induced resistance expression (Walters et al., 2011). Similar results were found in tomato cultivars treated with BABA (Sharma et al., 2010).

To conclude, the biological and functional character of an elicitor may play an essential role in the efficacy of induced plants resistance. BTH, (2R,3R)butanediol or PC1 showed different activation modes inducing resistance in the same host plant (Cortes-Barco et al., 2010). Such findings indicate that chemical elicitors might determine which genes or pathways will be activated.

CONCLUSION

The *in vitro* antifungal activity of different Palestinian herbal plant extracts and local commercial essential oils has proved the effectiveness of extract components such as coridothyme, salvia, varthemia against fungal growth. Moreover, the EOs application on wheat and barley cultivars showed varied induced responses according to the POX and PPO levels in each cultivar. For example, oregano, clove or lavender and pomegranate, common yarrow or chamomile oil effectively induced resistance indicator enzymes in wheat and barley.

To conclude, using EOs as plant resistance elicitors is promising for the loose smut disease management in barley and wheat. It can be considered a novel and risk-free biocontrol agent for plant disease control, intensifying crops production under a reduced need for synthetic chemicals.

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REFERENCES

Aditya S, Hegde GM (2020): Induction of defense enzymes in groundnut upon treatment with bioagents and botanicals. Journal of Pharmacognosy and Phytochemistry, 9, 180–183.

Almagro L, Gomez Ros LV, Belchi-Navarro S, Bru R, Ros Barcelo A, Pedreno MA (2008): Class III peroxidases in plant defense reactions. Journal of Experimental Botany, 60, 377–390. doi: 10.1093/jxb/ern277.

Aman M, Rai VR (2015): Antifungal activity of fungicides and plant extracts against yellow sigatoka disease-causing *Mycosxaerella musicola*. Current Research in Environmental and Applied Mycology, 5, 277–284. doi: 10.5943/cream/5/3/11.

Ayoub M, Niazi AU (2001): Control of wheat rust by leaves extract of poisonous phanergamic plants. Journal of Biological Sciences, 1, 490–491. doi: 10.3923/jbs.2001.490.491.

Behbahani BA, Shahidi F, Yazdi FT, Mortazavi SA, Mohebbi M (2017): Antioxidant activity and antimicrobial effect of tarragon (*Artemisia dracunculus*) extract and chemical composition of its essential oil. Journal of Food Measurement and Characterization, 11, 847-863. doi.org/10.1007/s11694-016-9456-3

Caetano AS, Chalfoun SM, Resende MV, Angelico CL, Santiago WD, Magalhaes ML, de Rezende DA, Soares LI, Nelson DL, Cardoso MG (2020): Chemical characterization and determination of *in vivo* and *in vitro* antifungal activity of essential oils from four Eucalyptus species against the

Hemileia vastatrix Berk and Br fungus, the agent of coffee leaf rust. Australian Journal of Crop Science, 14, 1379–1384. doi: 10.21475/ajcs.20.14.09.p2249.

Chittoor JM, Leach JE, White FF (1999): Induction of peroxidase during defense against pathogens. In: Datta SK, Muthukrishnan S (eds): Pathogenesis-related proteins in plants. CABI International, Wallingford, 171–193.

Cortes-Barco AM, Hsiang T, Goodwin PH (2010): Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (*2R, 3R*)-butanediol or an isoparaffin mixture. Annals of Applied Biology, 157, 179–189. doi: 10.1111/j.1744-7348.2010.00417.x.

Dann E, Diers B, Byrum J, Hammerschmidt R (1998): Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. European Journal of Plant Pathology, 104, 271–278. doi: 10.1023/A:1008683316629.

Datta SK, Muthukrishnan S (1999): Induction of peroxidase during defense against pathogens. In: Datta SK, Muthukrishnan S (eds): Pathogenesis-related proteins in plants. CRC Press, Boca Raton, 176–199.

Dey U, Harlapur SI, Dhutraj DN, Suryawanshi AP, Jagtap GP, Apet KT, Wagh SS (2013): Effect of fungicides, botanicals, bioagents and Indigenous Technology Knowledge (ITKs) on germination of *Uredinio* spores of *Puccinia sorghi in vitro*. African Journal of Agricultural Research, 8, 4960–4971. doi: 10.5897/AJAR2013.7770.

Flott BE, Moerschbacher BM, Reisener HJ (1989): Peroxidase isoenzyme patterns of resistant and susceptible wheat leaves following stem rust infection. New Phytologist, 111, 413–421. doi: 10.1111/j.1469-8137.1989.tb00704.x.

Fu ZQ, Dong X (2013): Systemic acquired resistance: turning local infection into global defense. Annual Review of Plant Biology, 64, 839–863. doi: 10.1146/annurev-arplant-042811-105606.

Gilchrist-Saavedra L (1997): Basidiomycetes. In: Gilchrist-Saavedra L, Fuentes Davila G, Martinez-Cano C, Lopez-Atilano RM, Duvieller E, Singh RP, Henry M, Garcia A (eds): Practical guide to the identification of selected diseases of wheat and barley. CIMMYT, Mexico, 18–24.

Jogaiah S, Satapute P, De Britto S, Konappa N, Udayashankar AC (2020): Exogenous priming of chitosan induces upregulation of phytohormones and resistance against cucumber powdery mildew disease is correlated with localized biosynthesis of defense enzymes. International Journal of Biological Macromolecules, 162, 1825–1838. doi: 10.1016/j.ijbiomac.2020.08.124.

Joseph B, Dar MA, Kumar V (2008): Bioefficacy of plant extracts to control *Fusarium solani* f. sp. *melongenae incitant* of brinjal wilt. Global Journal of Biotechnology and Biochemistry, 3, 56–59.

Kazemi N, Khavari-Nejad RA, Fahimi H, Saadatmand S, Nejad-Sattari T (2010): Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. Scientia Horticulturae, 126, 402–407. doi: 10.1016/j.scienta.2010.07.037.

Kerby K, Somerville S (1989): Enhancement of specific intercellular peroxidases following inoculation of barley with *Erysiphegraminis* f. sp. hordei. Physiological and Molecular Plant Pathology, 35, 323–337. doi: 10.1016/0885-5765(89)90037-4.

Kumar V, Tyagi D (2013): Antifungal activity evaluation of different extracts of *Bergenia stracheyi*. International Journal of Current Microbiology and Applied Sciences, 2, 69–78.

Lin J, Gong D, Zhu S, Zhang L, Zhang L (2011): Expression of PPO and POD genes and contents of polyphenolic compounds in harvested mango fruits in relation to benzothiadiazole-induced defense against anthracnose. Scientia Horticulturae, 130, 85–89. doi: 10.1016/j.scienta.2011.06.014.

Mohammadi M, Kazemi H (2002): Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Science, 162, 491–498. doi: 10.1016/S0168-9452(01)00538-6.

Onaran A (2016): *In vitro* antifungal activities of some plant extracts against plant pathogenic fungi in Turkey. Egyptian Journal of Biological Pest Control, 26, 111–114.

Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA (2014): Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology, 52, 347–375. doi: 10.1146/annurev-phyto-082712-102340.

Pinelo M, Manzocco L, Nunez MJ, Nicoli MC (2004): Solvent effect on quercetin antioxidant capacity. Food Chemistry, 88, 201–207. doi: 10.1016/j.foodchem.2004.01.034.

Pouresmaeil M, Nojadeh MS, Movafeghi A, Maggi F (2020): Exploring the bio-control efficacy of *artemisia fragrans* essential oil on the perennial weed *Convolvulus arvensis*: Inhibitory effects on the photosynthetic machinery and induction of oxidative stress. Industrial Crops and Products, 155, 112785. doi: 10.1016/j.indcrop.2020.112785.

Quijano CD, Wichmann F, Schlaich T, Fammartino A, Huckauf J, Schmidt K, Sautter C (2016): KP4 to control *Ustilago tritici* in wheat: Enhanced greenhouse resistance to loose smut and changes in transcript abundance of pathogen-related genes in infected KP4 plants. Biotechnology Reports, 11, 90–98. doi: 10.1016/j.btre.2016.08.002.

Rahber-Bhatti MH (1988): Antifungal properties of plant leaf decoctions against leaf rust of wheat. Pakistan Journal of Botany, 20, 259–263.

Rathod M, Das NA, Dhale D (2015): Antifungal activity of two medicinal plants against fungus *Candida albicans*. International Journal of Pharma and Biosciences, 6, 701–706.

Ravimannan N, Arulanantham R, Pathmanathan S, Niranjan K (2014): Alternative culture media for fungal growth using different formulation of protein sources. Annals of Biological Research, 5, 36–39.

Sales MD, Costa HB, Fernandes PM, Ventura JA, Meira, DD (2016): Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pacific Journal of Tropical Biomedicine, 6, 26–31. doi: 10.1016/j.apjtb.2015.09.026.

Schilling L, Matei A, Redkar A, Walbot V, Doehlemann G (2014): Virulence of the maize smut *Ustilago maydis* is shaped by organ-specific effectors. Molecular Plant Pathology, 15, 780–789. doi: 10.1111/mpp.12133.

Scorzoni L, Benaducci T, Almeida AM, Silva DH, Bolzani VD, Gianinni MJ (2007): The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp. and *Cryptococcus* sp. Brazilian Journal of Microbiology, 38, 391–397. doi: 10.1590/S1517-83822007000300001.

Scott IM, Samara R, Renaud JB, Sumarah MW (2017): Plant growth regulator-mediated anti-herbivore responses of cabbage (*Brassica oleracea*) against cabbage looper *Trichoplusia ni* Hübner (Lepidoptera, Noctuidae). Pesticide Biochemistry and Physiology, 141, 9–17. doi: 10.1016/j.pestbp.2016.10.005.

Shabana YM, Abdalla ME, Shahin AA, El-Sawy MM, Draz IS, Youssif AW (2017): Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*. Egyptian Journal of Basic and Applied Sciences, 4, 67–73. doi: 10.1016/j.ejbas.2016.09.002.

Sharma K, Butz AF, Finckh MR (2010): Effects of host and pathogen genotypes on inducibility of resistance in tomato (*Solanum lycopersicum*) to *Phytophthora infestans*. Plant Pathology, 59, 1062–1071. doi: 10.1111/j.1365-3059.2010.02341.x.

Tolman JH, McLeod DG, Harris CR (2004): Cost of crop losses in processing tomato and cabbage in southwestern Ontario due to insects, weeds and/or diseases. Canadian Journal of Plant Science, 84, 915–921. doi: 10.4141/P03-002.

Ton J, Van Pelt JA, Van Loon LC, Pieterse CM (2002): Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. Molecular Plant–Microbe Interactions, 15, 27–34. doi: 10.1094/MPMI.2002.15.1.27.

Tucci M, Ruocco M, De Masi L, De Palma M, Lorito M (2011): The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. Molecular Plant Pathology, 12, 341–354. doi: 10.1111/j.1364-3703.2010.00674.x.

Uma K, Huang X, Kumar BA (2017): Antifungal effect of plant extract and essential oil. Chinese Journal of Integrative Medicine, 23, 233–239. doi: 10.1007/s11655-016-2524-z.

van der Wolf JM, Michta A, van der Zouwen PS, de Boer WJ, Davelaar E, Stevens LH (2012): Seed and leaf treatments with natural compounds to induce resistance against *Peronospora parasitica* in *Brassica oleracea*. Crop Protection, 35, 78–84. doi: 10.1016/j.cropro.2012.01.008.

Walters DR, Havis ND, Paterson L, Taylor J, Walsh DJ (2011): Cultivar effects on the expression of induced resistance in spring barley. Plant Disease, 95, 595–600. doi: 10.1094/PDIS-08-10-0577.

Wilcoxson RD (1996): Loose smut. In: Nielsen J, Thomas P (eds): Bunt and smut diseases of wheat: concepts and methods of disease management. CIMMYT, Mexico, 33–47.

Zhang Z, Bi Y, Ge Y, Wang J, Deng J, Xie D, Wang Y (2011): Multiple pre-harvest treatments with acibenzolar-s-methyl reduce latent infection and induce resistance in muskmelon fruit. Scientia Horticulturae, 130, 126–132. doi: 10.1016/j.scienta.2011.06.024.

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