Natural Induced Resistance in Barley and Wheat Using Palestinian Endogenous Plant Extracts Against Loose Smut (*Ustilago nuda* and *Ustilago tritici*) Disease.

By:

Bushrah Karsou'

Supervisor:

Dr. Rana Samara

This Thesis was Submitted in Partial Fulfillment of the Requirements for the Master's Degree of Science in:

Agricultural Biotechnology

Deanship of Graduate Studies

Palestine Technical University-Kadoorie

January, 2019

تحفيز مقاومة النبات طبيعيا بواسطة مستخلصات نباتية فلسطينية لمكافحة مرض التفحم المكشوف في محصولي القمح والشعير

الطالبة:

بشرى كرسوع

المشرف:

الدكتورة رنا سمارة

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في:

التكنولوجيا الحيوية الزراعية

عمادة الدراسات العليا

جامعة فلسطين التقنية–خضوري

كانون الثاني، 2019

COMMITTEE DECISION

This thesis/dissertation (Natural Induced Resistance in Barley and Wheat Using Palestinian Endogenous Plant Extracts Against Loose Smut (*Ustilago nuda* and *Ustilago tritici*) Disease) was successfully defended and approved on 15/Jan./2019.

Examination committee **Signature**

- Dr. Rana Samara, (Supervisor) _____

Assoc. Prof. / College of Agricultural Sciences and Technology, Palestine Technical University-Kadoorie (PTUK).

- Dr. Raed Alkowni, (Member) _____

Assoc. Prof. / Faculty of Science, An-Najah National University.

- Dr. Yamen Hamdan, (Member) _____

Assoc. Prof. / College of Agricultural Sciences and Technology, Palestine Technical University-Kadoorie (PTUK).

خضوري -جامعة فلسطين التقنية

نموذج التفويض

- أنا بشرى رشيد مصطفى كرسوع، أفوض جامعة فلسطين التقنية خضوري بتزويد نسخ من رسالتي / أطروحتي للمكتبات والمؤسسات أو الهيئات أو الأشخاص عند طلبهم حسب التعليمات النافذة في الجامعة.

التوقيع:

التاريخ: 15/Jan./2019

Palestine Technical University-Kadoorie

(PTUK)

Authorization form

I, Bushrah Rasheed Mustaffa Karsou', authorize the PTUK to supply copies of my thesis / dissertation to libraries or establishments or individuals on request, according to the university of PTUK regulations.

Signature: _____

Date: 15/Jan./2019

Natural Induced Resistance in Barley and Wheat Using Palestinian Endogenous Plant Extracts Against Loose Smut (*Ustilago nuda* and *Ustilago tritici)* Disease

By:

Bushrah Karsou'

Supervised by:

Dr. Rana Samara

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في: التكنولوجيا الحيوية الزراعية.

This thesis was submitted in partial fulfillment of the requirements for the master degree in: Agricultural Biotechnology.

Deanship of Graduate Studies	عمادة الدراسات العليا
PTUK	جامعة فلسطين التقنية –خضوري
January/ 2019	كانون الثاني/ 2019

Natural Induced Resistance in Barley and Wheat Using Palestinian Endogenous Plant Extracts Against Loose Smut (*Ustilago nuda* and *Ustilago tritici)* Disease.

Dedication

I dedicate this thesis to the science and scientists, especially those interested in plants and environment fields, to Farmers, students, dreamers and ambitions, my University (PTUK), my Family and to myself.

Last but not least dedicate this to the spirit of my father in law (Faisal Abudullah) and my grandmother (Hossen Abu lymonah) who gone forever away from our eyes, my love and misses are beyond words. May Allah (SWT) grant you Jannah Firdaws, Amen.

Acknowledgements

A grateful acknowledge to ICARDA for providing the wheat and barley cultivars seeds used in this study. Also to Dr. Khaled Sawalha for providing the Palestinian herbal plants materials which are used in this study. To Palestinian Agricultural Academic Cooperation Project (PAAC) and Palestinian American research center (PARC) for their foundation.

I would like to thank all those contributed to this thesis. My great thanks is for my supervisor Dr. Rana Samara for her supervision, encouragement, guidance and help throughout this study. My thanks are also expressed to my university PTUK especially to all faculty members of Agricultural Science and Technology Department. A lot of thanks for the head of KARC Laboratory Dr. Nawaf Abu-Khalaf and the technician Basima Abu Rumaila for their help.

I want to thank my family, especially my parents the reason of what I reach today. To my brothers Firas and his wife Ala', Bashar and his wife Sawsan, Ammar and his wife Majd, and Mohammad, to my sisters Jehad, Noura, Duaa and Waad, to my beloved husband Bahaa' and to my family in law Hussneya, Diya', Tasneem, Taqwa, Yaqeen and Balsam, to my nephews Tala, Rasheed, Daysam, Asia, Sama, Malak, Estya and Lana. To my future children and also to my friends especially Dr. Nesreen tamimi and Dr. Basma Damiri, for cheering me up when things did not work. Thanks for

your continuous care and support. Thank you for inspirit me. Thank you for your understanding, patience and love.

First and foremost, my gratitude is to my God for reconcile me in my life. Thank you, lord, for always being there for me. Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout.

Table of content

List of tables

List of figures

List of abbreviation

Abbreviation	Description
%	Percent
µg	Microgram
µL	Microliter
µM	Micromolar
ANOVA	Analysis of variance
bp	Base pair
℃	Celsius degree
cm	Centimeters
C	Water control treatment
CRBD	Complete randomized block design
CTAB	Cetyltrimethylammoniun bromide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DW	Distilled water
EDTA	Ethylene diamine tetra acetic acid
ET	Ethylene
F	Fungal inoculation treatment
g	Gram
h	Hour/ hours
H_2O_2	Hydrogen peroxidase
HR	Hypersensitive response
ICARDA	International Center for Agricultural Research in the Dry Areas
IPM	Integrated pest management
ISR	Induce systemic resistance
JA	Jasmonic acid
K_3PO_4	Potassium phosphate
KARC	Kadoorie Agricultural Research Center
L	Liter
M	Molar
Mg	Milligram
Mg SO_4	Magnesium sulfate
Min	Minute/ Minutes
mL	Milliliter
mM	Millimolar
Mm	Millimeter
NaCl	Sodium chloride
NaOCl	Sodioum Hypochloride
NH_4OAC	Ammonium acetate
nm	Nanometer

NPK	Nitrogen: phosphorus: potassium
PAAC	Palestinian Agricultural Academic Cooperation Project
PARC	Palestinian American research center
PCD	Programmed cell death
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PGPF	Plant growth promoting fungus
PGPR	Plant growth promoting Rhizobacteria
Ph	Acidity or basicity
PO	Plant extracts and commercial oils
POX	Guaiacol-Peroxidase
PPO	Polyphenol oxidase
PR	Pathogen Related
Psi	Pounds per square inch
PTUK	Palestine Technical University - Kadoorie
PVP	Polyvinylpyrrolidone
Rnase	Ribonuclease
rpm	Round per minute
s	Second/ Seconds
SAR	Systemic acquired resistance
SAS	Statistical analysis software
SDS	Sodium dodecyl sulfate
sp.	Species
Std	Standard deviation
TAE	Tris base, acetic acid and EDTA
TE	Tris- HCl and EDTA
Tris- HCl	Tris hypochlorite
U	Unit
UV	Ultra violet
W	Wound treatment
w: v	Weight per volume
WHO	World health organization
x	Times

Natural Induced Resistance in Barley and Wheat Using Palestinian Endogenous Plant Extracts Against Loose Smut (*Ustilago nuda* and *Ustilago tritici)* Disease.

By: Bushrah Karsou'

Supervised by: Dr. Rana Samara

Abstract

Many plant diseases attack Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). They are considered as the most produced crops world wild. Loose smut *Ustilago tritici* Pers. on wheat and *Ustilago nuda* Jens. Rostr. On barley are very common in the West Bank, Palestine. Fungicides are commonly used in controlling loose smut disease, but searching for an alternative and safe methods are conducted in order to innovate new techniques contributing toward sustainable agriculture production and food security.

In this study different Palestinian plant extracts were tested against the fungi *Ustilago* sp.. Moreover, they were tested for their potentiality in inducing resistance pathways on four different cultivars of wheat and barley. Measuring two common enzymes indicator such as guaiacol Peroxidase (POX) and Polyphenol oxidase (PPO) expressed in plants after being induced physically or chemically. In vitro antifungal activity of the plant extracts on the fungal growth was carried out.

Results showed that 70% of the plant extracts have antifungal activity against *Ustilago* sp.. Coridothyme extracts was ranked first with 61% growth inhibition among all. Results also revealed that some plant extracts have significantly increased the impact on POX and/ or PPO compared to the control among all wheat and barley cultivars tested. Oregano, Clove or Lavender and Pomegranate, Achillce or Cammomile oil have effective induction for resistance indicator enzymes in wheat and barley, respectively.

This study, investigated the role of endogenous Palestinian plant extracts in inducing wheat and barley resistance systems against loose smut disease. In-order to alternate chemical control with fungicides.

Key Words: Wheat, Barley, POX, PPO, *Ustilago tritici*, *Ustilago nuda*, Plant Extracts, Induce Resistance.

1. Introduction

1.1 General introduction

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are members of poaceae family belonging to poales order. They are considered the most produced crops worldwide as food source for both human and animals. Moreover they have abundant benefits on human health and diet. Wheat and barley contain basic nutrients like carbohydrate, proteins and fibers, they also contain lipids, vitamins especially B type, phytochemicals, antioxidants, minerals such as iron and others in smaller portions (Ames & Rhymer, 2008; Newton et al., 2011; Shewry & Hey, 2015). Cultivated Area and production of field crops in the Palestinian territory are (245414 Dunums) and (444404 metric ton), respectively according to Palestinian Central Bureau of Statistics (PCBS) (2012). Wheat and barley ranked the first and the second crops by their cultivated area and production. Wheat is holding (211052 Dunums) and the production is (39431 metric ton). Barley is holding (108888 Dumms) and the production is (13895 metric ton) (PCBS, 2007).

Many plant diseases caused by fungi, bacteria and viruses may infect these crops, causing damages and economic losses. One of these diseases is loose smut of wheat caused by *Ustilago tritici* Pers. and in barley caused by *Ustilago nuda* Jens. Rostr. Loose smut is very common in these crops growing in North-Western and in West Bank, Palestine. Although this disease causes only minor losses in the yield, it is a potential threat to

international trade of commercial grain and food security policy; due to reducing seed quality and quantity. The percentage of infection equals loss in yield, and most of the returns of the yield goes into production cost, even 1-2 percent (%) infection could decrease framers revenue by 5-20% (Wilcoxson, 1996). Infected plants only show symptoms when the fungus is colonized and spread, that makes it hard to recognize infected plants from healthy one, which could lead to enormous yield losses in the absence of convenient management practices (Kassa, Menzies, & Mc Cartney, 2014; Koch, Zink, Büssers, & Gossmann, 2013; Wunderle, Leclerque, Schaffrath, Slusarenko, & Koch, 2012). The symptoms of loose smut fungi are easy to recognize because of producing brown to black spores foremost in grains. Also, the teliospores are the most remarkable taxonomic merit of ustilaginaceae family of fungi which are belonging to basidiomycota phylum (Wilcoxson, 1996).

Tolman, McLeod, and Harris, (2004) reported that when pests were not controlled, crop yields were reduced by at least 80%. Moreover, traditional methods that were attempt to control loose smut disease are many such as: breeding methods, heat treatment and chemical fungicides, which are still difficult to obtain and are considered time consuming (Wilcoxson, 1996).

Biological control, integrated pest management (IPM) and transgenic crops, are the alternative to chemical pest control. However, transgenic crops may

not be allowed in certain jurisdictions (Burketova, Trda, Ott, & Valentova, 2015).

European Union and global organizations are working on reducing the use of pesticide worldwide by looking for alternative ways to control plant diseases. Also, in particular view the difficulty in identifying infected plant with loose smut disease which is related to the fact that infected plants appears healthy until they reach maturity and show disease symptoms. Thus using chemical control for loose smut disease is difficult. Integration of plant induced resistance is one of the new safe innovated techniques to protect crops from disease infections, prevent crop losses and contribute to sustainable agriculture production and food security (Vincelli, 2016; Walters, Ratsep, & Havis, 2013).

This recent innovation in crop protection which may replace fungicides by stimulating the plant immune system post treating target plants with biotech or a-biotech agents (Burketova et al., 2015). Treatment of plants with variable natural elicitors can induce plant resistance and enhance disease control associated with increased activities of the plant defense related enzymes and phenolic contents. Such researches are getting more attention and growing interest (Boughton, Hoover, & Felton, 2006; Walters et al., 2013).

1.2 Aims of project

Therefore, the aims of this project were to:

- Investigate the potential antifungal activity of different Palestinian herbal plant extracts and local commercial oils (PO) in vitro against *Ustilago* sp. fungi.
- Determine the response of wheat and barley cultivars to post fungal inoculation and, PO treatment, by trying to evaluate the induction kind of pathogen related proteins.
- Introduce safe alternative controlling method to shrink the usage of synthetic pesticides by investigating the role of Palestinian PO treatment in inducing plants own resistance system.

2. Literature review

2.1 Phytopathogens insight

Phytopathogens researches on fungal, viral and bacterial pathogens are extensive. Also different diseases controlling methods had been reviewed many times.

Phytopathogenic fungi are considered as the major plant disease agents in plants. Causing serious quality and quantity problems during plant development, post harvesting and storage. This lead to massive yield reduction and economics losses (Anderson et al., 2004; Dellavalle et al., 2011).

2.2 Controlling methods against fungal phytopathogens

Controlling strategies against phytopathogenic fungi are extensively dependent on synthetic fungicide. Recently more public concerns were increased about the impact of using synthetic fungicides on human and animal health, environment and agroecosystem. Agrochemicals usage are increasingly restricted and regulated due to their negative effects, especially after their extensive usage and rising of crop races resist to fungicides (Harris, Renfrew, & Woolridge, 2001). Scientists are looking for alternative methods other than agrochemicals (De Cal, Sztejnberg, Sabuquillo, & Melgarejo, 2009; Kalidindi et al., 2015; M, Reuveni, Agapov, & R, Reuveni, 1995).

2.3 Antimicrobial activity of deferent bioagents

Antimicrobial and bioactivity of different bioagents were studied thoroughly, such as microbial extracts, organic materials, essential oils, plant seed oils (Deepthi et al., 2007; Madhusudhan, Nalini, Prakash, & Shetty, 2005; Madhusudhan et al., 2011; Prasad, Shankar, Kumar, Shetty, & Prakash, 2007) pure secondary metabolites, new synthesized molecules and plant extracts. These naturally agents could provide a rich characterized range of varied active compounds that could be potential bio-pesticide against insect pests and diseases (Mabona, Viljoen, Shikanga, Marston, & Van Vuuren, 2013; Nazzaro, Fratianni, De Martino, Coppola, & De Feo, 2013; Runyoro, Matee, Ngassapa, Joseph, & Mbwambo, 2006).

2.4 Herbal plants usage

Many studies reported that, plant extracts have been used as a medicinal treatment for many infectious diseases in human and animals (Bussmann, Glenn, Meyer, Kuhlman, & Townesmith, 2010; Demo & Oliva, 2008). Herbal folk medicine and remedies were used as analgesic, antidepressant, antispasmodic, antimicrobial، astringent, diuretic, stimulant, laxative, hepatic diseases, in external use for inflammatory disorders, burns, eczema, healing wound, sedative, digestive and others (Dellavalle et al., 2011). According to World Health Organization (WHO) there is an increment on herbal plant usage, whereas 80% of world's population use herbal plants for primary health care purposes (Mazid, Khan, & Mohammad, 2012).

Cowan (1999) reported that plant tissues hold the active natural compounds that acting against the disease casual agents. Bioactivity of plants extracted by different solvents (Aman & Rai, 2015) from different sources such as roots, leaves, seeds or flowers have been investigated by many researchers for their inhibitory potential against broad range of phytopathogens (Dellavalle et al., 2011).

Many of different important organic compounds, pharmaceuticals and pesticides have been derived from plant sources (Varma & Dubey, 1999), could be a potential for alternative agrochemicals usage (Cushnie & Lamb, 2005; Raj, Vennila, Aiyavu, & Panneerselvam, 2009).

Plant extracts was not only reported as an effective bioagents against wide range of phytopathogens (Baraka, Radwan, Shaban, & Arafat, 2011; Chaudhary & Chaudhari, 2013; Joseph, Dar, & Kumar, 2008; Mariita et al., 2011; Shabana et al., 2017), but also it was reported as elicitor or bio-stimulant (Calvo, Nelson, & Kloepper, 2014). It could induce host resistance pathways (Walters & Heil, 2007). Hence plant extracts has been tested against *Erwinia amylovora* (Burrill) on its resistance induction impact (Zeller, 2006). Also *Hedera helix* (L.) extract was reported to induce resistance on apple root stocks (Bengtsson et al., 2009).

2.5 Plant defense response

2.5.1 Plant induced resistance

Recently, plant induced resistance term has been raised by many researchers as a new approach in disease management (Benhamou, Lafontaine, & Nicole, 1994; Benhamou, Kloepper, Quadt-Hallman, & Tuzun, 1996). A better understanding of plant signaling pathways triggered naturally by herbivores and/ or pathogens could be the potential and the innovated strategies for plant disease control (Karban & Kuc, 1999; Vallad & Goodman, 2004; Walters et al., 2013).

Treatment of plants with wide range of various agents such as cell wall fragments, plant extracts and synthetic chemicals that mimic a pathogen attack could induce plant resistance to subsequent pathogen attack and against a broad spectrum of pathogen (da Rocha & Hammerschmidt, 2005; Lyon, 2007).

Plants developed a variety of strategies to protect themselves against pathogens and environmental stress (Walling, 2000). In contrast pathogens have produced compounds that have the ability to suppress plant defense mechanisms. If pathogens exceed the first line of defense, which is the physical barriers including plant cell wall, plasma membrane, waxy cuticle, bark and others, plants may use the chemical barriers as defense responses including production of toxins, antimicrobial compounds or enzymes, different secondary metabolites, rapid release of reactive oxygen species,

phytoalexin accumulation and enhanced levels of pathogen related (PR) proteins. One of the defense strategies in plants is the hypersensitive response (HR), which is induced by recognize pathogen effectors by plants gene products. HR is compromised by programmed cell death (PCD) at infection site (Edreva, 2004). As a result the rest of plant became protected from pathogen access. Mobile signals are transmitted from induction site to plant tissues to protect it from the invading pathogen and or any other pathogens. This phenomenon is called induce resistance such as systemic acquired resistance (SAR) and induce systemic resistance (ISR) (Herman & Williams, 2012).

2.5.2 Systemic acquired resistance (SAR)

SAR is a form of induced resistance that is salicylic acid (SA) dependent pathway (Uknes et al., 1992; Ward et al., 1991). SA is suggested to stimulate expression of PR genes coding for proteins that have critical role in pathogens resistance. SAR could be triggered naturally in plant after pathogen attack. Also it is very effective against a broad range of pathogens, long lasting and could prime plants for successive infection (Deepak, Raj, Umemura, Kono, & Shetty, 2003; Hammerschmidt & Yang-Cashman, 1995; Kessler & Baldwin, 2002; Nandakumar, Babu, Viswanathan, Raguchander, & Samiyappan, 2001; Van Loon, Bakker, & Pieterse, 1998; Walling, 2000).

2.5.3 Induced systemic resistance (ISR)

ISR is the second main type of induce resistance pathways, it also provide protection to the plant from pathogens, but it developed mainly by the colonization of plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungus (PGPF) (Van Loon et al., 1998) and it's mediated by a jasmonate (JA) - and ethylene (ET)-dependent pathway (Knoester, Pieterse, Bol, & Van Loon, 1999; Pieterse et al., 1998). Also, there is a web and too disharmony between the regulation mechanisms in SAR and ISR resistance pathways (Vallad & Goodman, 2004).

3 Material and method

3.1 Fungal methods

3.1.1 Media preparation

The fungi used in this study were cultured on potato dextrose agar (PDA) media prepared according to the manufacturer instructions available on bottle (BD, difco^{tm}, Ref: 254920), autoclaved, poured into sterile petri dishes and stored at 4℃ for further use (Rathod, Das, & Dhale, 2015).

3.1.2 Fungal isolation and maintenance

Loose smut fungus was isolated from infected wheat sample collected in 2015 in PTUK. Samples were rinsed three times with sterile DW, then it was incubated for 2 min in 0.05% sodium hypochlorite (NaOCl) and the second 2 min it was incubated in 70% ethanol as reported by (Gilchrist-Saavedra, 1997). A small portion of sample were placed over fresh prepared PDA media, sealed with parafilm, labeled and incubated at 37℃. Purified isolated of this fungus was sustained on PDA media every 2-3 weeks (Ravimannan, Arulanantham, Pathmanathan, & Niranjan, 2014).

3.1.3 Pathogen identification

3.1.3.1 Pathogen morphological identification

Fungal identification are conducted based on spore shape and color, and also, the dikaryotic mycelium growth after 2 weeks of incubation. Fungal swap was mounted in water and then observed by bright field microscope using an inverted microscope– Optika XDS-2 Trinocular (AIPTEK international

GmbH, Italy). Images were recorded with an AIPTEK HD1080P digital camera (AIPTEK international GmbH, Germany).

3.1.3.2 Molecular identification of *Ustilago*. sp.

3.1.3.2.1 Deoxyribonucleic acid (DNA) isolation

DNA was extracted from isolated fungi following a modified cetyl-trimethyl-ammonium bromide (CTAB) protocol (Reineke, Karlovsky, & Zebitz, 1998; Rogers & Bendich, 1994). Briefly, 50-100 mg of five days grown fungi were scraped from PDA surface media using clean and sterile scalpel, then transferred to sterile 1.5 milliliter microfuge tube containing around 100 mg of sterile sand. 500 microliter (µL) extraction buffer (100 millimolar (mM) Tris hypochlorite (Tris- HCl, 10 m Methylene-diamine-tetra-acetic acid (EDTA), 2% sodium dodecyl sulfate (SDS), 100 microgram (µg)/ mL proteinase K and 1% B-mercapto-ethanol) were added to each tube. Tubes content were homogenized using pellet pestles cordless motor (Sigma-aldrich, Z359971-1EA) with sterilized tips (sigma-aldrich, Z359947). Then, microfuge tubes were incubated at 60℃ water bath for 1 h with shaking and swirling every 5-10 min. After that, salt concentration was adjusted to 1.4 molar (M) by adding 200 µL of 5 M sodium chloride (NaCl). Then 1: 10 of the total volume was added from 10% prepared CTAB solution (Sigma, St. Louis, MO). Then, tubes were incubated for 10 min at 65℃. After finishing this step, one volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and mixed gently by inverting tubes. Then tubes were

incubated at 0℃ for 30 min and centrifuged at 12000 round per minute (rpm) for 10 min at 4℃. Supernatant was carefully transferred into new 1.5 mL microfuge tube followed by adding 1:2 of the transferred volume from 5 M Ammonium acetate (NH_4OAc) and mixing gently and incubated in ice for 1 h. Tubes were then centrifuged at 12000 rpm for 15 min at 4℃.

The resulted supernatant was transferred to new tube again. Ribonuclease (RNase) was added to have a final concentration of 0.02 unite (U) g/ mL and mixing gently. This step was followed by adding 0.55 final volume from cold isopropsnol for precipitation step. Tubes were centrifuged for 5 min at 10000 rpm to collect DNA. Resulting pellets were re-suspended in 200 µL Tris-HCl and EDTA (TE) buffer and 20 µL of 3M NaOAc, pouvoir hydrogene (ph) 7. Then, 2.5 of the final volume was added from 75% cold ethanol solution. Tubes were centrifuged for 5 min at 10000 rpm. At the end, pellets were dried at room temperature around 30 min. and eluted in 100 µL TE buffer before being confirmed using gel electro-phoresis. Tubes were kept at -20℃ for further uses (Mohammadi & Kazemi, 2002, Resende et al., 2002).

3.1.3.2.2 Polymerase Chain Reaction (PCR)

PCR was performed using an automated veriti 96 well thermal cycler (applied biosystem company) device. Amplification was carried out with *Taq* DNA polymerase following the manufacture's setup (Hy laboratories Ltd company, HTD0078). PCR reaction volume of 20 µL contained 4 µL

from 20 mM magnesium sulphate ($MgSO_4$), 2 µL from 10 times (x) *Taq* reaction buffer, 0.4 µL from 10 mM deoxyribo-nucleotide triphosphate (dNTP), 0.2 µL from 5 U/ µL *Taq* DNA polymerase, 0.2 µL from 100 micromolar (µM) each primer, 2 µL of isolated saved DNA samples and 11 µL from ultra-pure dnase free water (Biological Industries, 1710266). The amplification reaction with the pair of primers 26S1 (5-GAGTAGAGGT-CGCGAGAGAGCAG-3)/ 26S2 (5-GATTGGTCG-TTGTGTGTCACC-3) were carried out under the following conditions. An initial step of 95℃ for 5 min, followed by 30 cycles of 95℃ for 1 min, annealing step of 54℃ for 2 min and 72℃ for 2 min. Then, a final elongation step of 72℃ for 10 min (Basse, Stumpferl, & Kahmann, 2000).

3.1.3.2.3 DNA Gel electrophoresis

Isolated DNA and PCR products were visualized by running on 0.8% and 1.5% weight per volume (w: v) prepared agarose gel, respectively. In which, it was prepared by completely dissolving 0.8 g and 1.5 g of agarose in 100 mL of 0.5x Tris base, acetic acid and EDTA (TAE) buffer by heating using microwave. Then, 8 µL of 1000 x gel red stain (Biotium GelRed, 41003- F) was added and lifted to cool. The agarose was poured in a tray of (Submarine Horizontal) type electrophoresis system (Helixx, Mupid- exU). 5 µL of the DNA with 1 µL of a bromophenol blue loading dye were loaded carefully in a well of solidified gel. First well in gel is used to load a DNA ladder ready to use 3 µL from 1 kilo base pairs (kpb) and 100 base pairs (pb) for DNA

isolation and PCR assay respectively. After electro-phoresis ran DNA bands were viewed under ultra violet (UV) illuminator device (UVITEC, Cambridge) and gel documentation system (Meyers, Sanchez, Elwell, & Falkow, 1976).

3.2 Host plant methods

3.2.1 Plant sample collection

Four wheat and barley cultivars listed in (Table 1) used in this study. Seeds for each cultivar were provided from International Center for Agricultural Research in the Dry Areas (ICARDA) and had been stored dry in refrigerator.

3.2.2 Viability test for seed

Germination ability of seeds was tested before being used in this study. Where, 2 replicates of 50 seeds of each cultivar were placed on moist filter paper in 90 millimeter (mm) petri dishes with labeling and incubated at 26 ± 2°C. Germination percentage were calculated by counting number of total germinated seeds in each plate after 72 h over number of total seeds multiply by 100 (Siddiqui, Bhardwaj, Khan, & Meghvanshi, 2009)

Table 1. List of provided wheat and barley cultivars from ICARDA.

No.	Name	Lab ID
Wheat cultivars		
1	Local wheat	W1
2	H. Safraa'	W2
3	Kahlaa'	W3
4	Nab Al- Jamal	W4
Barley cultivars		
1	Local barley	B1
2	Barley 17/14	B2
3	Ryhan	B3
4	Barley akad 76	B4

3.2.3 Host plant maintenance

All cultivars seeds were propagated in glasshouse at PTUK (Figure 1) under adjusted temperature 28 ± 2°C, humidity 60% and fertilized weekly after pass two foliar stage with Nitrogen: Phosphate: Potassium(NPK) (20: 20: 20) fertilizer and watered as required. Soil combination used in this study contained an autoclaved mixture of 2:1:1 volume ratio of Patmos: vermiculite: sand.

Figure 1. Propagated cultivars at glass house conditions in PTUK. A: wheat propagated cultivars, B: barley propagated cultivars.

3.2.4 Host fungal infection

Fungal inoculation experiment was conducted and maintained under glasshouse condition in 9 centimeter (cm) pots with 4- 6 seeds with 3 replication for each treatment following Complete randomized block design (CRBD). The treatment details were:

- plants inoculated (injected) with pathogen (F),
- plants that were injected with needle without Pathogen (W), and
- plants not inoculated with pathogen and not injected with needle (C)

Plant inoculated assay had been described by (Furumo & Furutani, 2008). Each cultivar seedling were injected at three leaf stage with pathogen by injection method (Quijano et al., 2016; Schilling, Matei, Redkar, Walbot, & Doehlemann, 2014). 400 µL from 1.5 $x10^8$ spore/ mL *Ustilago* sp. suspension was syringed in each seedling tissue at points into center of

injection site which was chosen approximately 1 cm above soil (Figure 2). All treatments were maintained in glasshouse condition.

Figure 2. Host plantlet injection with *Ustilago* sp. fungi at three foliar stage.

3.2.4.1 Assessment of fungal infection on enzymes activation of host plants

Peroxidase (POX) and polyphenol oxidase (PPO) activities were measured spectrophotometrically using (Hach Lange DR6000 UV-VIS Spectro-photometer, Germany) for each wheat and barley cultivar with 3 replicate for each treatment after 48 h of inoculation based on method of (JOE & Muthukumaran, 2008; Scott, Samara, Renaud, & Sumarah, 2017). POX and

PPO activity was measured for each -80℃ frozen sample by protein extraction steps, which are: placing 3 g of leaves for each treatment of each cultivar in 2 mL eppendorf tube prior to homogenization in 1.25 µL of 0.1M potassium phosphate (K_3PO_4) buffer (ph 7, containing 7% (w: v) polyvinyl-pyrrolidone (PVP)) using pellet pestles cordless motor with 1.5 mL sterilized tips. Each homogenated extract was transferred to a new centrifuge tube. Then 400 µL of 10% solution of Triton x-100 was added with mixing vigorously around 10 second (s). Tubes were centrifuged for 8000 rpm for 15 min using (Hettich® MIKRO 200/200R centrifuge, Z652121 SIGMA). Then, each supernatant was used immediately as enzyme source for enzyme activity assay. All of these steps were maintained in ice condition. In order to determine POX activity 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 1 mL of fresh prepared 5 mM guaiacol with 0.02 mM hydrogen peroxide (H_2O_2) dissolved in 0.1M K_3PO_4 buffer ph 8 (Rai, 2015). For PPO assay 10 µL of enzyme extract was added to 2 mL disposable cuvette containing 500 µL of fresh prepared 10 mM catechol dissolved in 0.1M K_3PO_4 buffer ph 8. Changes in absorbance were measured at 470 nanometer (nm) for 30 s at room temperature using spectrophotometer device. Enzyme specific activity for both enzymes were reported as Absorbance/min/mg of fresh tissue weight (Boughton et al., 2006; Furumo & Furutani, 2008; Thaler, Stout, Karban, & Duffey, 1996).

3.3 Herbal plant experiments

3.3.1 Herbal plant collection and Extraction

Different medicinal plants and commercial prepared oils (PO) shown in (Table 2) were collected in 2015-2016. Plants tissues were dried at room temperature in controlled condition in Kadoorie Agricultural Research Center (KARC) laboratories. Dried parts were grand to fine powder using mechanical mixer (Kumar & Tyagi, 2013). Then the obtained stock powders were kept in air tight glass bottles at 4℃ for further uses (Aman & Rai, 2015). Methanol extracts were prepared as described by (Mohana & Raveesha, 2007). 20 g grounded material of each plant were extracted with methanol ratio 1:3 w: v at high temperature 80℃ with agitation. Each homogenate was filtered through (Macherey-Nagel Filter Paper). Resulted aqueous solution was transferred to rotator evaporator device (Shanghai shen sheng technology R201BC rotating rotary evaporator original R205D 2l) and adjusted at 65℃ to be evaporated and to concentrate the active material and essential oils. Extract for each plant was collected after solvent evaporation (Kumar & Tyagi, 2013). Then dark air tight bottles was stored at 4℃ before being used in further experiments (Mohana & Raveesha, 2007).

Table 2. List of medicinal plants and commercial available oils used in study.

No.	Scientific name	English common name	Arabic common name	Family	Part used
Palestinian herbal plants					
1	*Ajuga iva*	Bugleweed	الجعدة	Lamiaceae	Leaf
2	*Ambrosia artemisiifolia*	Common ragweed	عشبة الوادي	Asteraceae	Leaf
3	*Ambrosia artemisiifolia*	Common ragweed	عشبة الوادي	Asteraceae	Root
4	*Artemisia arborescens*	Tree wormwood	الشيح	Asteraceae	Leaf
5	*Salvia fruticose*	Greek oregano	الميرمية	Lamiaceae	Leaf
6	*Thymus*	Green Thyme	الزعتر الأخضر	Lamiaceae	Leaf
7	*Thymus citriodorus*	Lemonthyme	زعتر الليمون	Lamiaceae	Leaf
8	*Coridothymu scapitatus*	Coridothyme	الزعتر الفارسي	Lamiaceae	Leaf
9	*Achillea millefolium*	Common yarrow	القيصوم	Asteraceae	Leaf
10	*Varthemia persica*	Foldy look	الكتيلة، صفيرا	Asteraceae	Leaf
11	*Matricaria recutita*	Camomile	البابونج	Asteraceae	Leaf
12	*Origanum vulgare*	Oregano	الأوريجانو	Lamiaceae	Leaf

Commercial oils					
13	*Rosmarinus officinalis*	Rosemary	اكليل الجبل	Lamiaceae	Prepared oil
14	*Syzygium aromaticum*	Clove	القرنفل	Myrtaceae	Prepared oil
15	*Sinapis arvensis*	Mustard	الخردل	Brassicaceae	Prepared oil
16	*Cinnamomum camphora*	Camphor	الكافور	Lauraceae	Prepared oil
17	*Punica granatum*	Pomegranate	الرمان	Lythraceae	Prepared oil
18	*Mentha spicata*	Mint	النعنع	Lamiaceae	Prepared oil
19	*Salvia fruticose*	Greek oregano	الميرمية	Lamiaceae	Prepared oil
20	*Lavandula spica*	Lavender	الخزامى	Lamiaceae	Prepared oil

3.3.2 PO bioactivity against *Ustilago*.sp.

Bio-efficacy of PO was tested against smut fungi in vitro. Plate- hole diffusion technique (Janssen, Scheffer, & Svendsen, 1987; Sales, Costa, Fernandes, Ventura, & Meira, 2016; Scorzoni et al., 2007) was applied to screening antifungal activity of 20 different PO under aseptic condition. 70 µL of $1.5x10^6$ spore/ mL fungal suspension, that was calculated using hemocytometer (ART.No.1280) (Gilchrist-Saavedra, 1997), were spread onto surface of prepared 60 mm PDA media. Then, 5 mm of soaked sterile disk filter papers in extract (for around 10 min in aseptic condition) were placed over the center of spread PDA media (3 replications were maintained for each PO treatment, control and solvent). In which, control treatment was done by soaking sterile filters in DW. Methanol soaked filter was conducted to check out the inhibitory effect of solvent on fungi. Petri dishes were parafilmed, labeled and incubated at 37℃ (Rathod et al., 2015). The antifungal activity was evaluated by measuring the diameter of inhibition (clear zone) of fungal growth formed around filter paper which considered as indicative of antifungal activity, after 24 h and 72h. Percent inhibition of fungal growth was calculated for each treatment using the formula: (I = C – T / C X 100) where I = Percent inhibition C = Fungal growth in control and T = Fungal growth in treatment (Aman & Rai, 2015; Janssen et al. 1987; Kumar & Tyagi, 2013).

3.3.3 Host plant treatment with PO

Effect of extracted plants and commercial oils was also tested on wheat and barley cultivars. This experiment was conducted and maintained under glasshouse condition following CRBD. 9 cm pots with 4 seeds with 3 replications for each PO treatment of wheat and barley cultivars. Treatment details were:

- 20 PO listed in (Table 2),
- 1st control (water) treatment, and
- 2nd control solvent application.

After plants get 3 to 4 leaf stage plantlet, leaves were dipping in PO, water and methanol (Cuthbertson et al., 2009). Leaves tissue sample from each treatment of cultivars post 48 h was collected, labeled and stored at -80℃ for leaf chemistry analysis.

3.3.3.1 Assessment of PO treatment on enzyme activity of host plant

POX and PPO activities were measured in collected samples. As describe in section 3.2.4.1.

3.4 Data analysis

Collection spread data sheets were analyzed using Analysis of variance (ANOVA) test using general linear models procedure (PROC GLM). Level of significance were determined by applying Duncan's multiple grouping test. On means and the standard deviation (Std) of 3 replicate readings at P=

0.05. All statistical analysis were performed using Statistical Analysis System (SAS) (SAS Institute 2008). Figures and diagrams were carried out using sigma plot system.

4 Results

4.1 Fungal identification

4.1.1 Pathogen microscopic identification

Morphological identification of the pathogen is presented in Figure 3. Black powdery grain in a smutted head (sooty) is composed of thousands of microscopic smut spores (Figure 3 A). Also the dikaryotic hayphal stage of fungi was detected under light microscope (Figure 3 B).

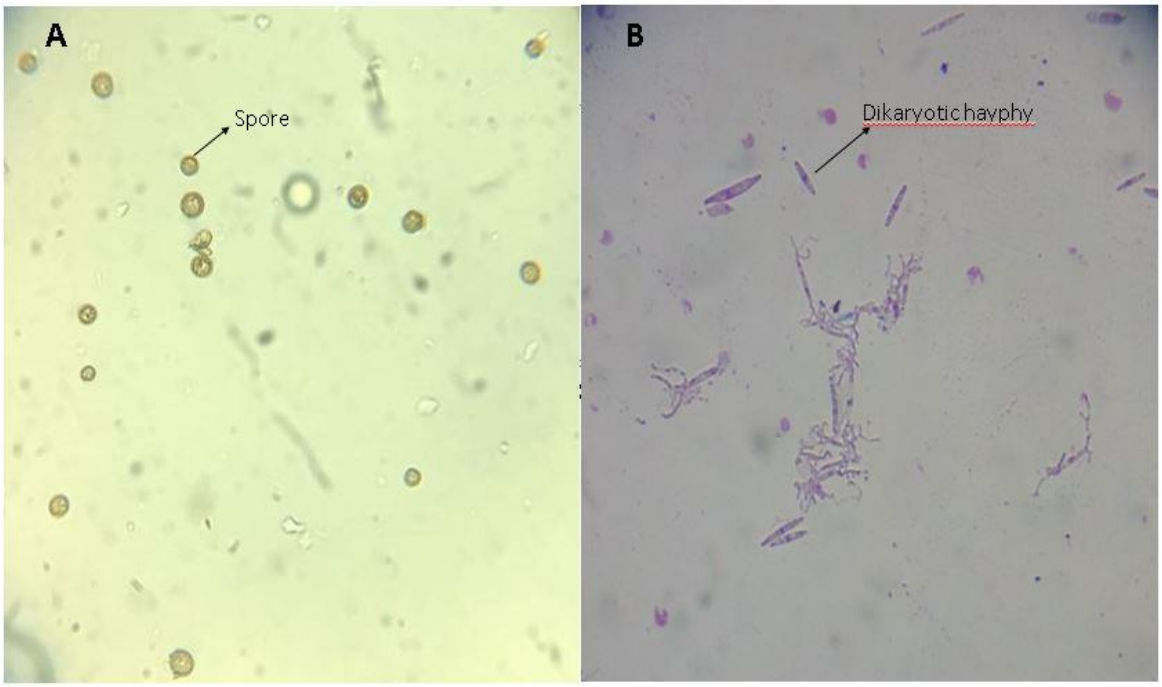

Figure 3. Microscopic image for A: diluted spores of *Ustilago* sp. B: dikaryotichayphe of *Ustilago* sp. fungi (1000x).

4.1.2 Total DNA Extraction

Identification and verification of *Ustilago* sp. isolates was also confirmed by the total genome using agarose gel electrophoresis (Figure 4 A), while, PCR products for *Ustilago* sp. isolated DNA using 26S primers set are presented in Figure 4 B. The presence of 1.5 Kpb bands in lane 1and 2

from 2 DNA isolated fungal samples, confirmed that these samples are belong to Ustilaginaceae family.

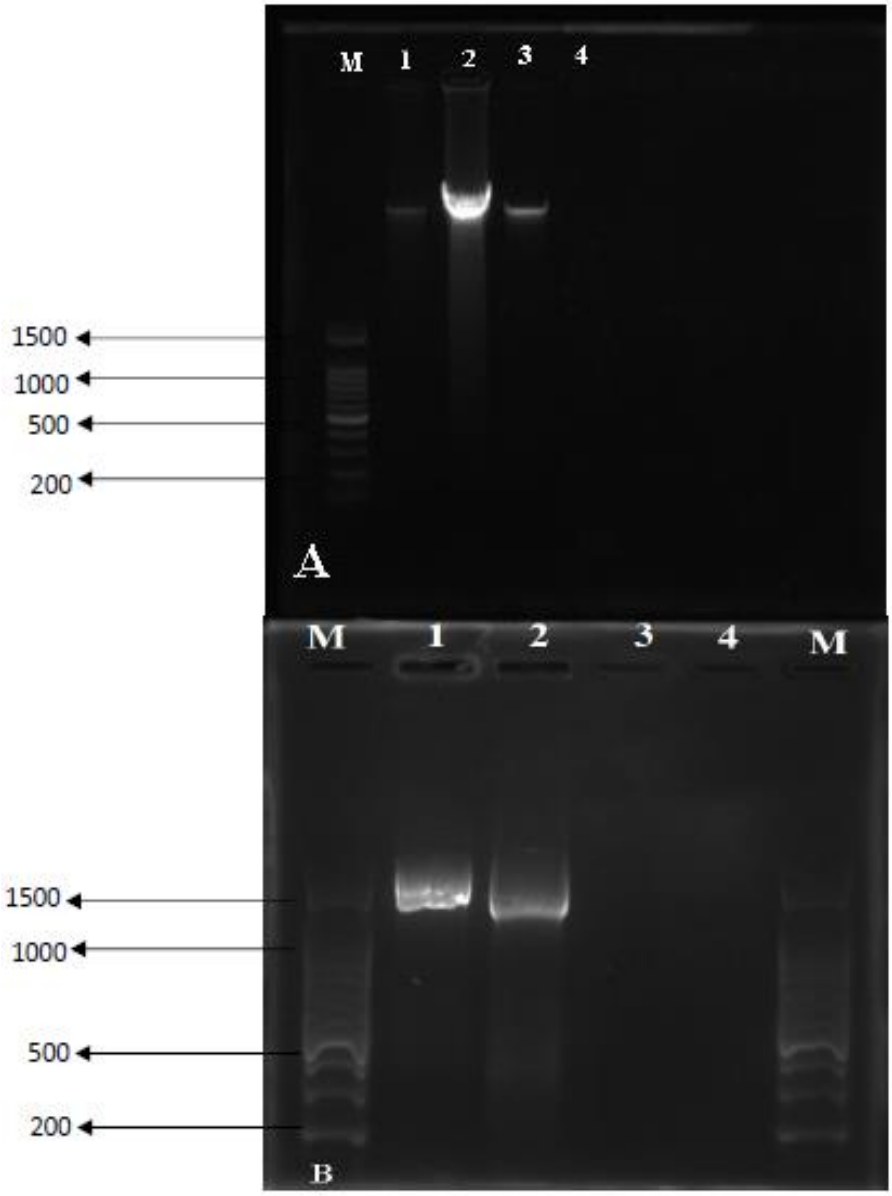

Figure 4. Agarose gel-electrophoresis of A: DNA extracted from fungal samples. B: PCR products amplified from *Ustilago* sp. DNA extract. M=100 bp DNA marker. Lane 1 and 2= *Ustilago* sp. fungi samples; Lane 3=other type of fungi as positive control, Lane 4= negative control.

4.2 Host plant method

4.2.1 Viability test for seed

Viability test (Figure 5) carried out resulted in 100% germination rate for both wheat and barley seed used in this study.

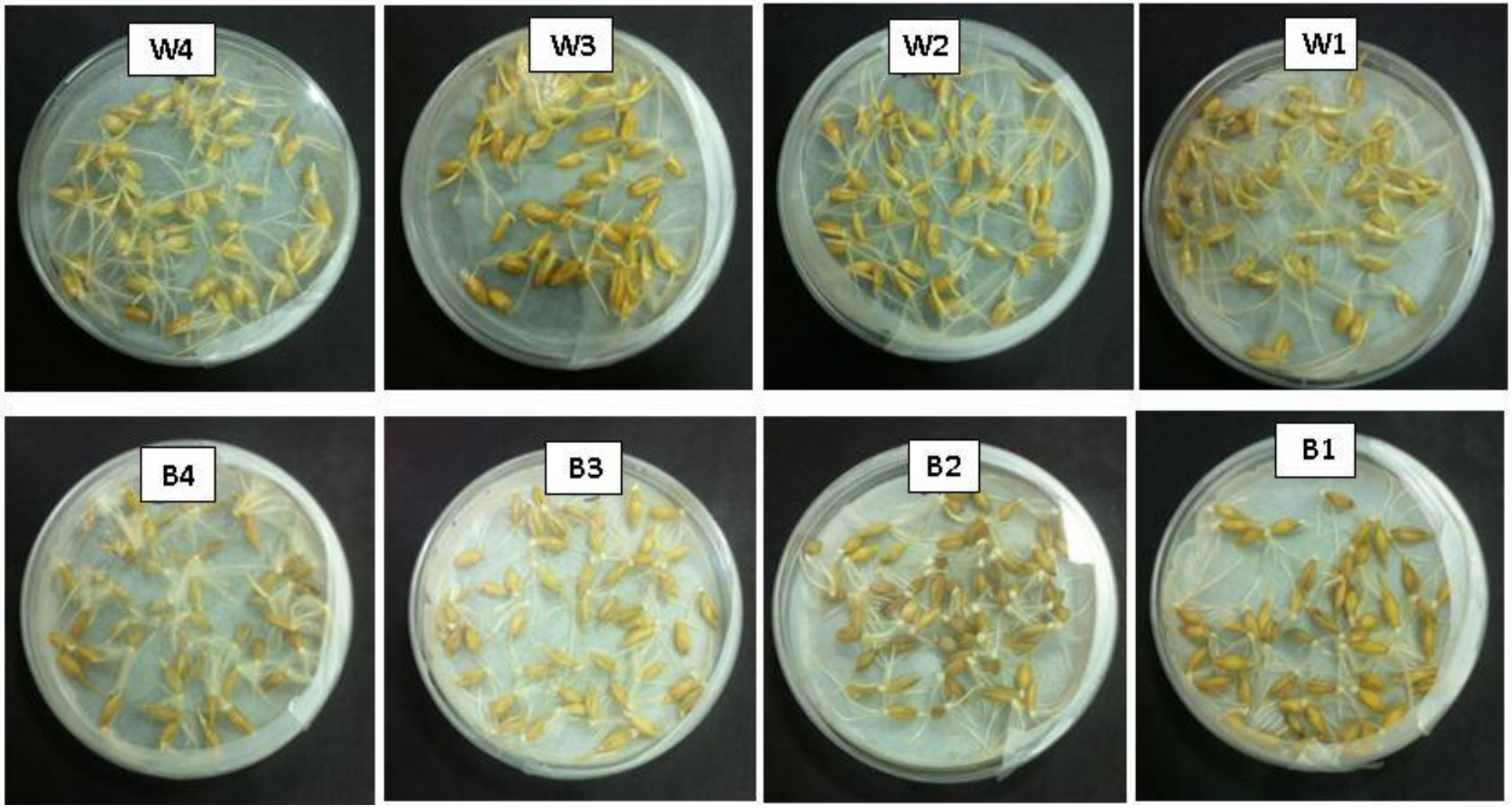

Figure 5. Wheat and barley cultivars germinated seeds after 72 h incubation at 26 ± 2°C

4.2.2 Effect of *Ustilago* sp. injection on host leaf enzymes

The results of PPO and POX specific activity post fungal inoculation treatment are significantly variable. PPO activity post fungal inoculation for wheat cultivars (Figure 6) recorded a significant high value of 0.185OD/min/mg in W3 cultivar compared with control value (0.132OD/min/mg), no significant impact of the fungal inoculation on the PPO level in W1, W2 and W4 cultivars.

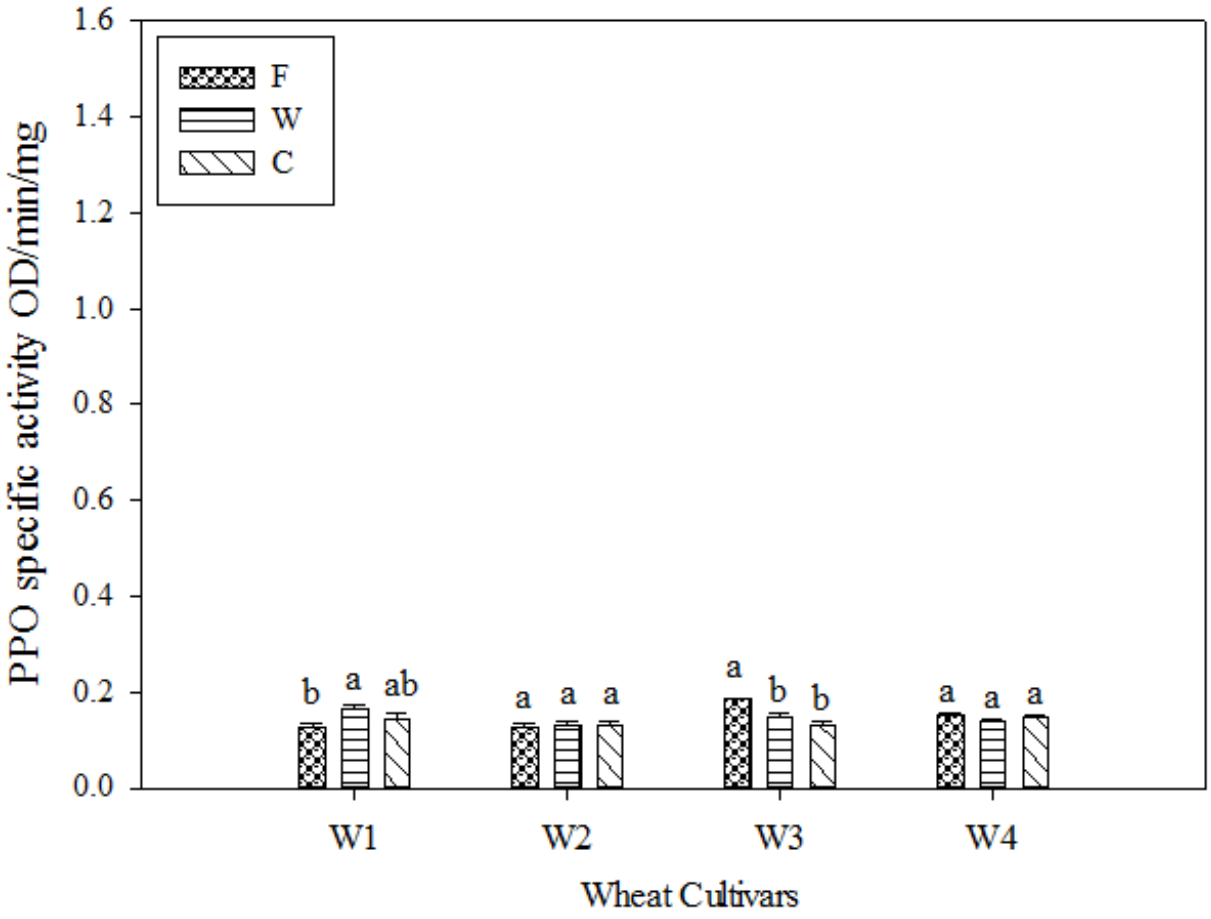

Figure 6. PPO specific activity in wheat cultivars leaves at 3 foliar stage after *Ustilago* sp. Inoculation. Each column represent mean and Std of 3 replicates. Similar latter represent same impact at P= 0.05.

Post fungal inoculation in barley cultivars, PPO level (Figure 7) was significantly lower in B1and B3 cultivars (0.035 and 0.143 OD/min/mg) compared with the control (0.079 and 0.171 OD/min/mg), respectively, No significant impact of the fungal inoculation on the PPO level in B3 and B4 cultivars.

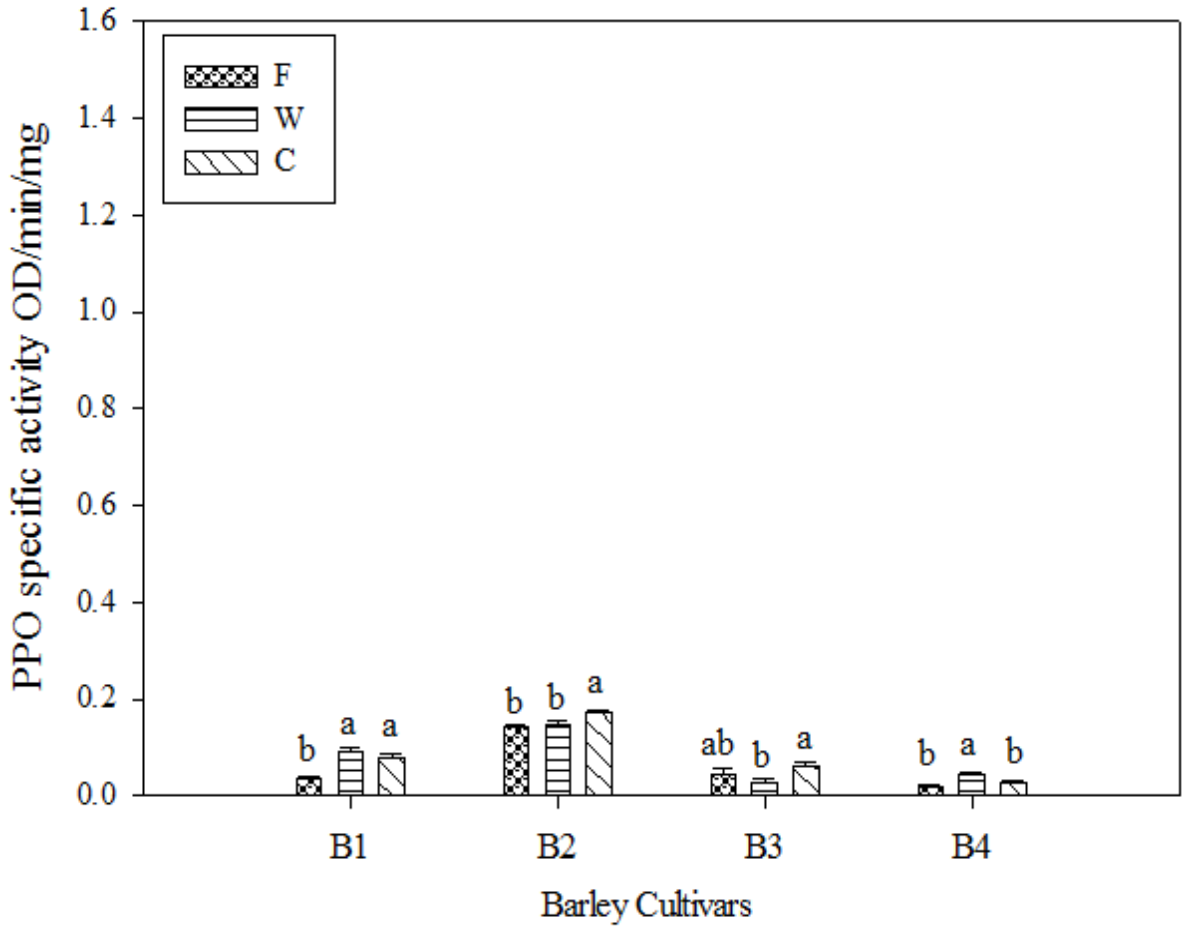

Figure 7. PPO specific activity in barley cultivars leaves at 3 foliar stage after *Ustilago* sp. Inoculation. Each column represent mean and Std of 3 replicates. Similar latter represent same impact at P= 0.05.

Results regarding to POX activity post fungal inoculation wheat cultivars (Figure 8) represent that all wheat cultivars were significantly higher than their each control sample (the control POX reading are 0.94, 0.94, 0.97 and 0.97 OD/min/mg).In which, W1and W2 cultivars had 1.06 times POX reading of the control, W3 had 1.03 times and W4 had 1.08 times the readings.

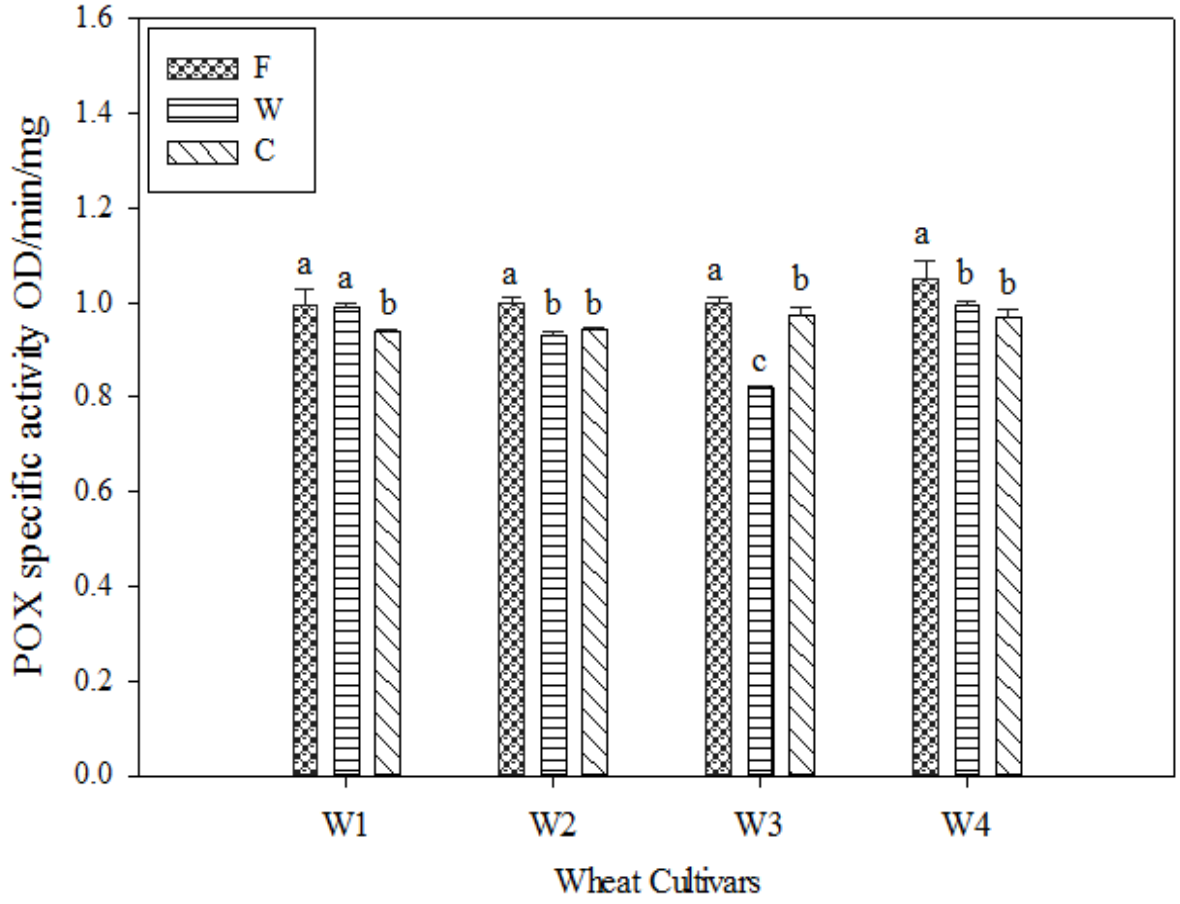

Figure 8. POX specific activity in wheat cultivars leaves at 3 foliar stage after *Ustilago* sp. Inoculation. Each column represent mean and Std of 3 replicates. Similar latter represent same impact at P= 0.05.

Post fungal inoculation in barley cultivars, POX level (Figure 9) was significantly lower (0.80, 1.28and 0.02 OD/min/mg) for B1, B2 and B4, respectively, according to their control values (0.87, 1.41and 0.87 OD/min/mg), POX level in B3 was significantly higher significant impact of the fungal inoculation on the POX level in B3 (1.05 OD/min/mg) cultivar compared with control reading (0.83 OD/min/mg)

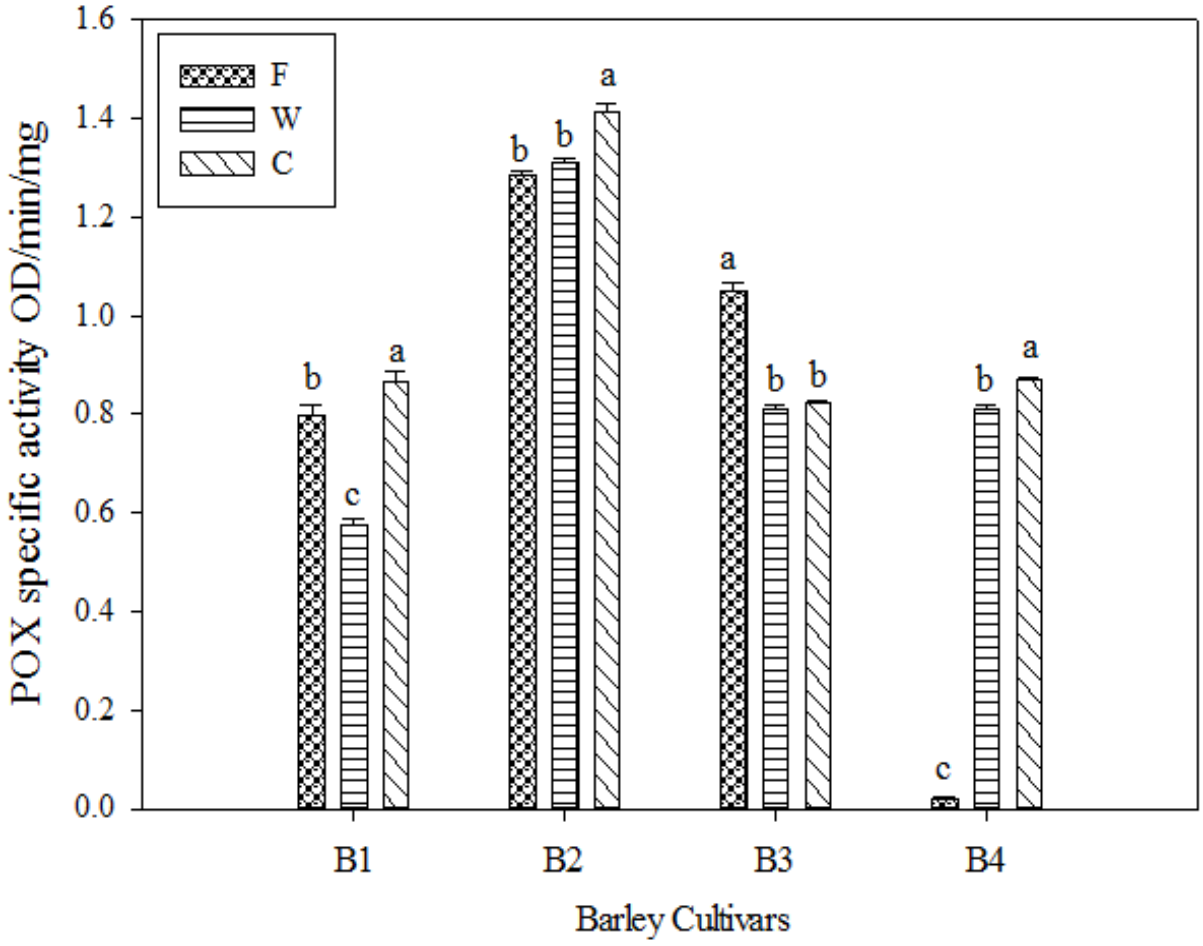

Figure 9. POX specific activity in barley cultivars leaves at 3 foliar stage after *Ustilago* sp. inoculation. Each column represent mean and Std of 3 replicates. Similar latter represent same impact at P= 0.05.

4.3 Plant extracts experiments

4.3.1 Antifungal activity of plant extracts against *Ustilago* sp.

Fungal growth inhibition % results (Figure 10) at 24 h (Figure 11) revealed that among all PO used in study, Coridothyme extract was the most effective one recording 59.0%. Followed by, Salvia extract recording more than 49%Also, Varthemia extract effect was the third one on growth inhibition with 43%. Lemonthyme, Ambrosia leaf, Ajuga, Clove, Atremesia, Green thyme, Ambrosia root, oil Salvia, Oregano, Achillce and Camomile were showed antifungal activity with percentage of inhibition equal 39.0, 38.3, 36.0, 35.7, 35.0, 33.0, 32.3, 31.7, 31.0, 29.7and 29.0, respectively. Whereas the oils: Lavender, Mint, Pomegranate, Cafour, Mustard and Rosemary did not showed any inhibition activity against *Ustilago* sp. when they are compared with water control.

Coridithyme is still the most effective extract at 72 h (Figure 12) recording 60.7% fungal growth inhibition. Followed by Salvia, Varthemia, Atremesia, Lemonthyme, Ambrosia root, Ambrosia leaf, Clove, Green thyme, oil Salvia, Camomile, Ajuga, Oregano and Achillce which were exhibited antifungal activity with inhibition percentage of 53, 48, 46.7, 42.0, 40.3, 40.3, 39.7, 39.0, 38.3, 35.0, 34.7 and 33.7, respectively. Whereas the oils: Lavender, Mint, Pomegranate, Cafour, Mustard and Rosemary did not showed any inhibition activity against *Ustilago* sp. when they are compared with water control.

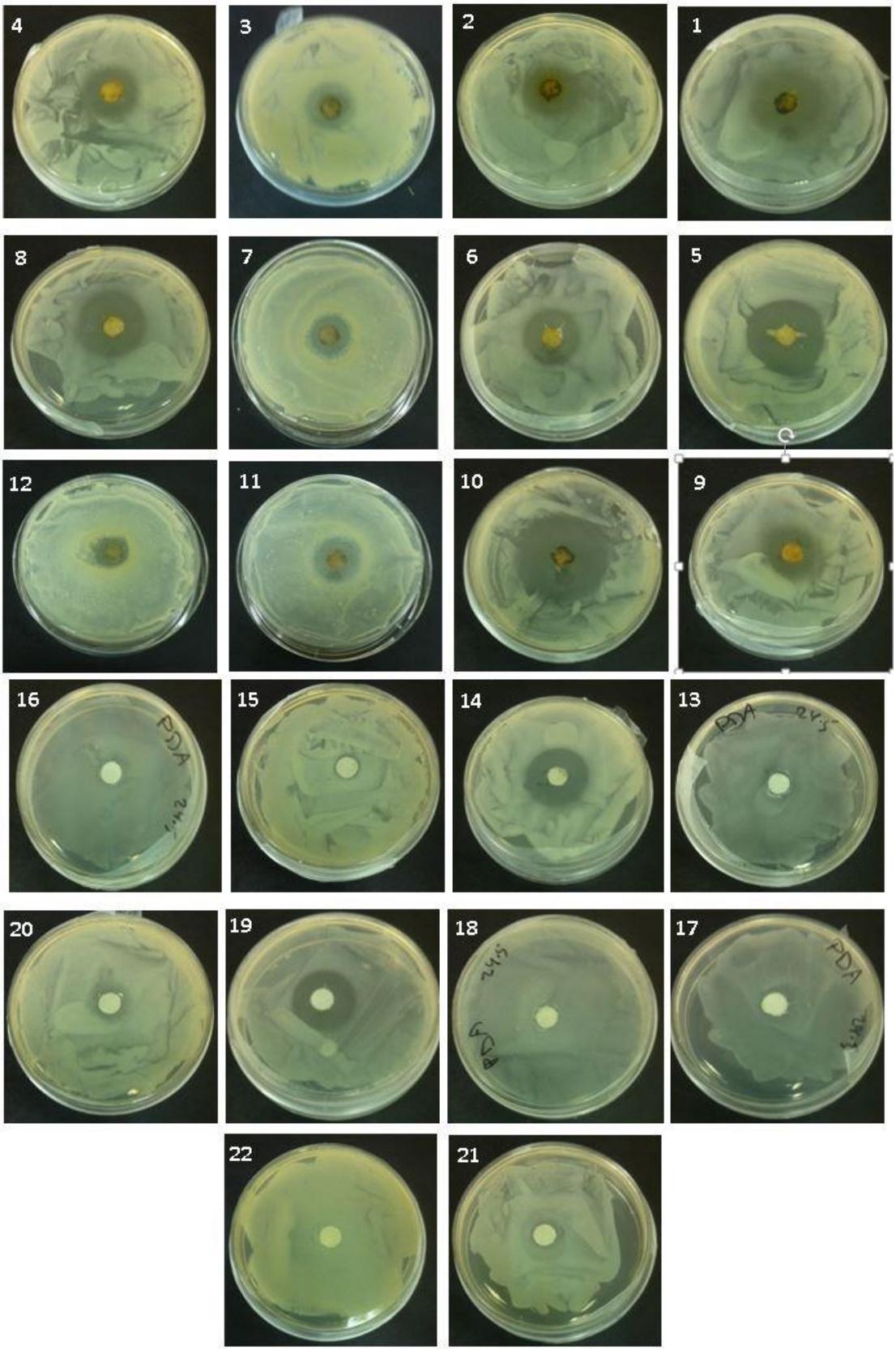

Figure 10. Inhibition zone in growth PDA media of *Ustilago* sp. fungi after PO application. Each number represent the PO items listed in Table 2, except no. 21 that considered as methanol control and no. 22 that considered as water control.

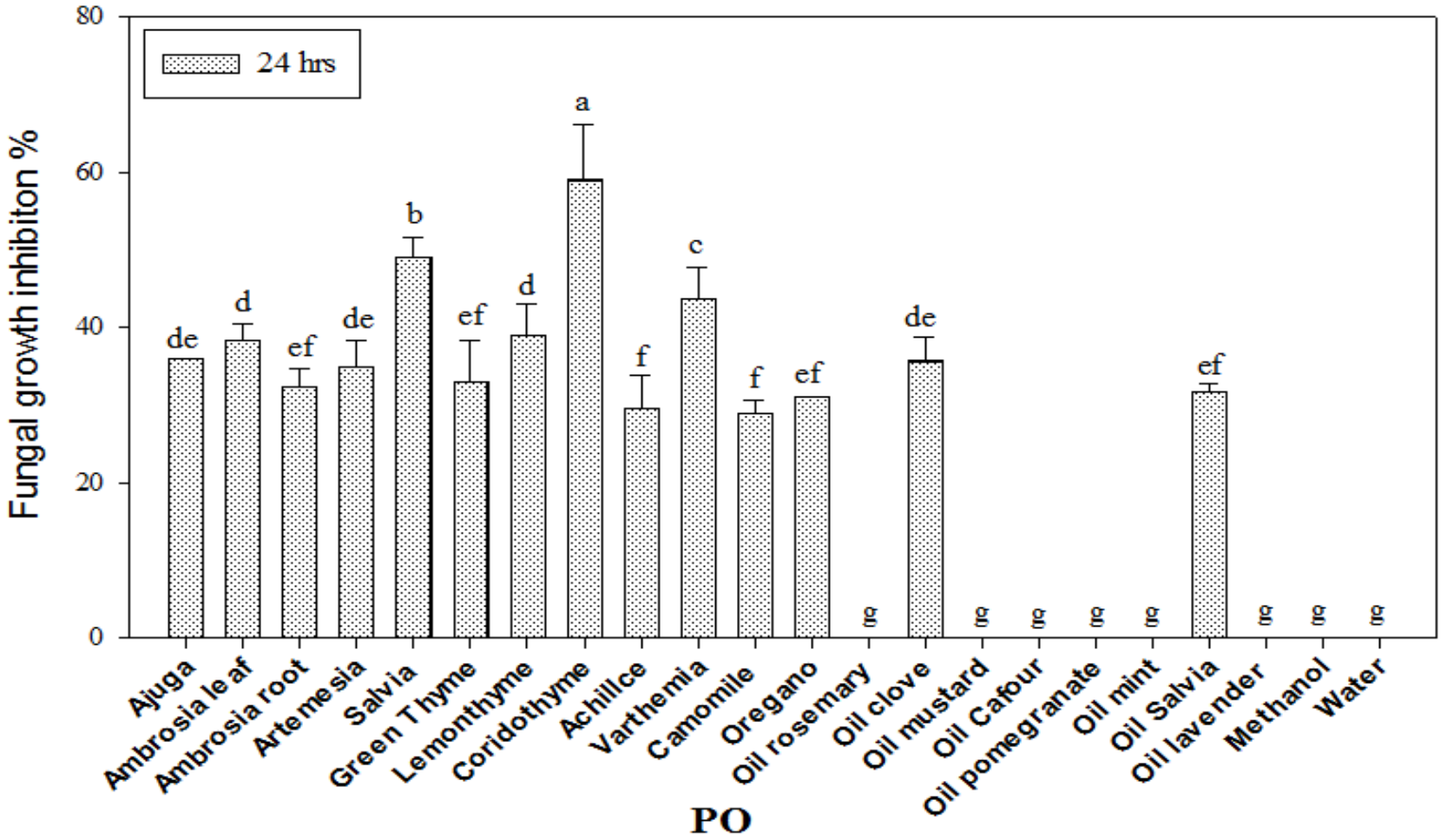

Figure 11. Fungal growth inhibition% efficacy of PO against *Ustilago* sp. fungi invitro after 24 h. Results represent mean and Std of 3 replicates. Similar latter represent same effect at P= 0.05

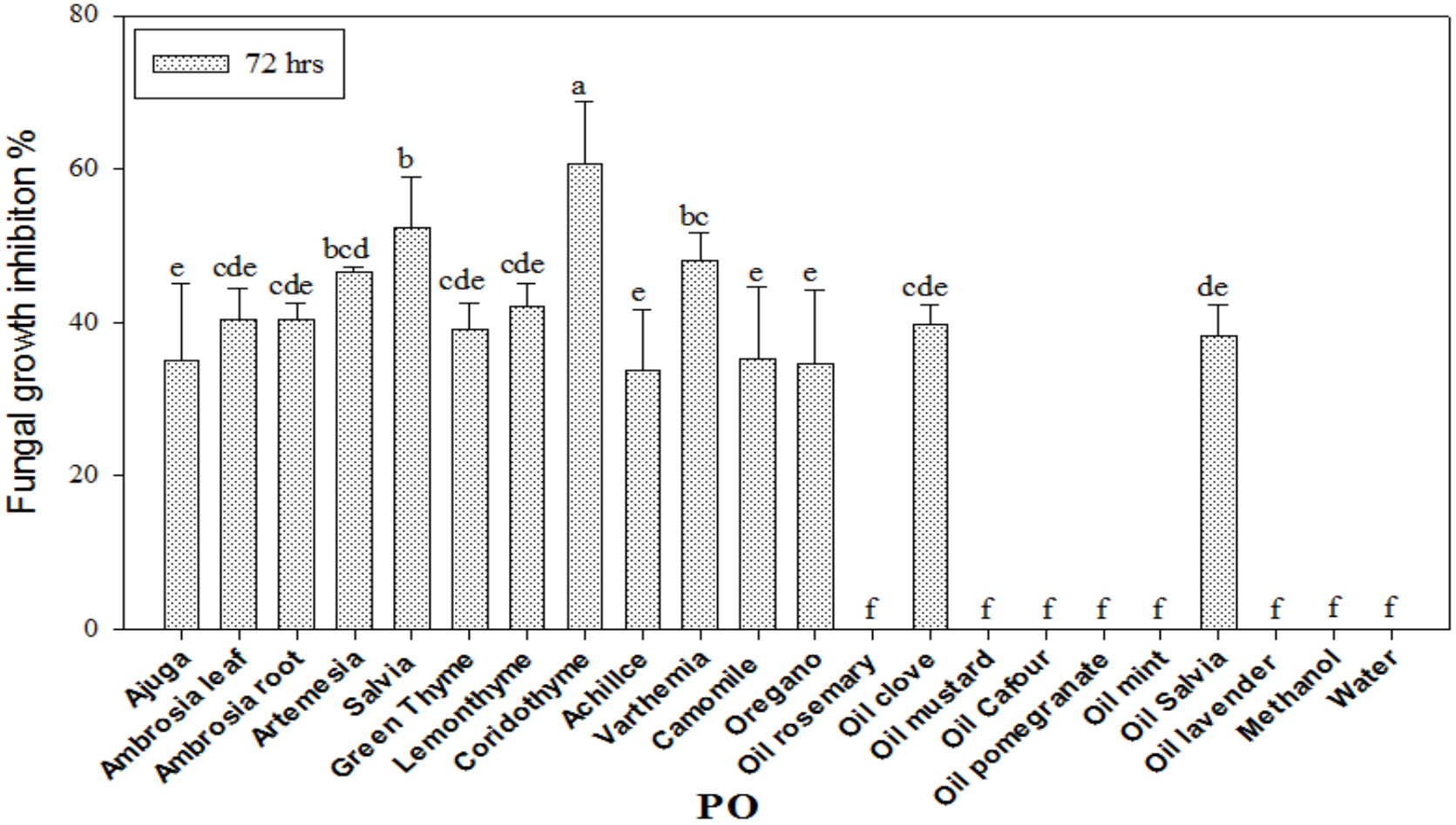

Figure 12. Fungal growth inhibition% efficacy of PO against *Ustilago* sp. fungi invitro after 72 h. Results represent mean and Std of 3 replicates. Similar latter represent same effect at P= 0.05.

4.3.2 Assessment of PO treatment on enzyme activity of host plant

1. PO treatment impact on PPO activity of wheat and barley cultivars leaves

PPO activity readings for wheat cultivars generally had increasing, similar and decreasing effect compared with water control at (p=0.05) post treatment with the PO mentioned in (Table.2).

Where, in W1 cultivar plants it had the maximum significant increment impact on PPO activity of 0.181 OD/min/mg compared with control activity reading of 0.121 OD/min/mg after being treated with oil Clove. Followed by oil Rosemary and Oregano which had an increasing effect on PPO level in W1 leaves by1.3 and 1.2 times, respectively. Conversaly, Achilce, Coridothyme, oil Salvia, Artemesia, Green thym, Lemonthyme, Camomile, Varthemia, Ambrosia root, oil Mustard, Salvia, Ajuga, Ambrosia leaf and oil Cafour had significant decreasing impact on PPO activity with reading ranged from 0.104 to 0.031 OD/min/mg. While oil Pomegranate and oil Mint had similar impact of control. Methanol treatment plants exhibited a little significant decreasing effect on PPO activity by 0.109 OD/min mg for W1 PPO activity which is illustrated in Figure 13.

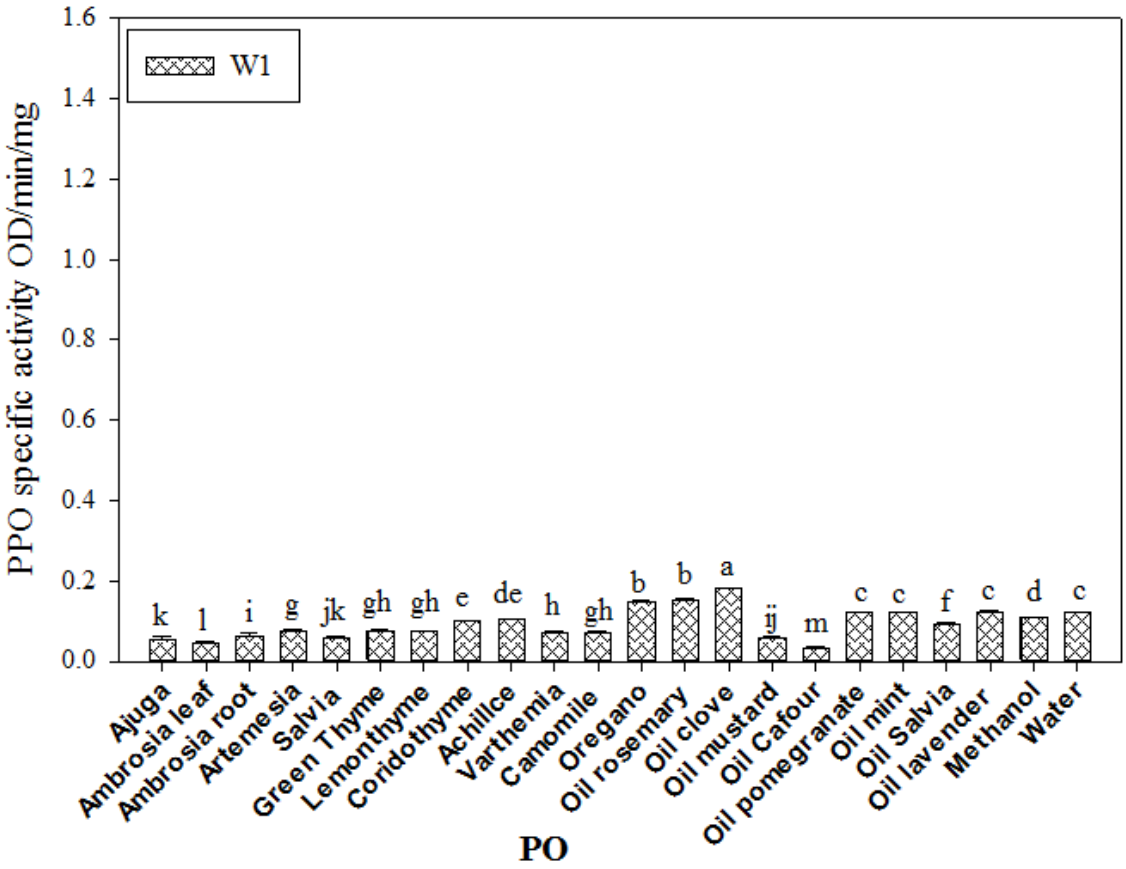

Figure 13. PPO specific activity in W1 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicates. Similar latter represent same effect at P= 0.05.

In W2 cultivar analyzed leaves (Figure 14) findings showed that oil Lavender treatment plants had significantly increased PPO activity by 5.5 times more than control activity value of 0.111 OD/min mg. Followed by, oil Salvia and oil Pomegranate which had significantly increased PPO levels by 2.2 and 2.1 times, respectively. While other PO had a significant decreasing impact on W2 cultivar's PPO activity with values ranged between 0.1- 0.04 OD/min/mg. Oil clove and oil Rosemary had no significant impact on PPO activity comparing to control. Meanwhile, methanol treatment on W2 leaves recorded a slightly high PPO activity value of 0.126 OD/min/mg compared with control.

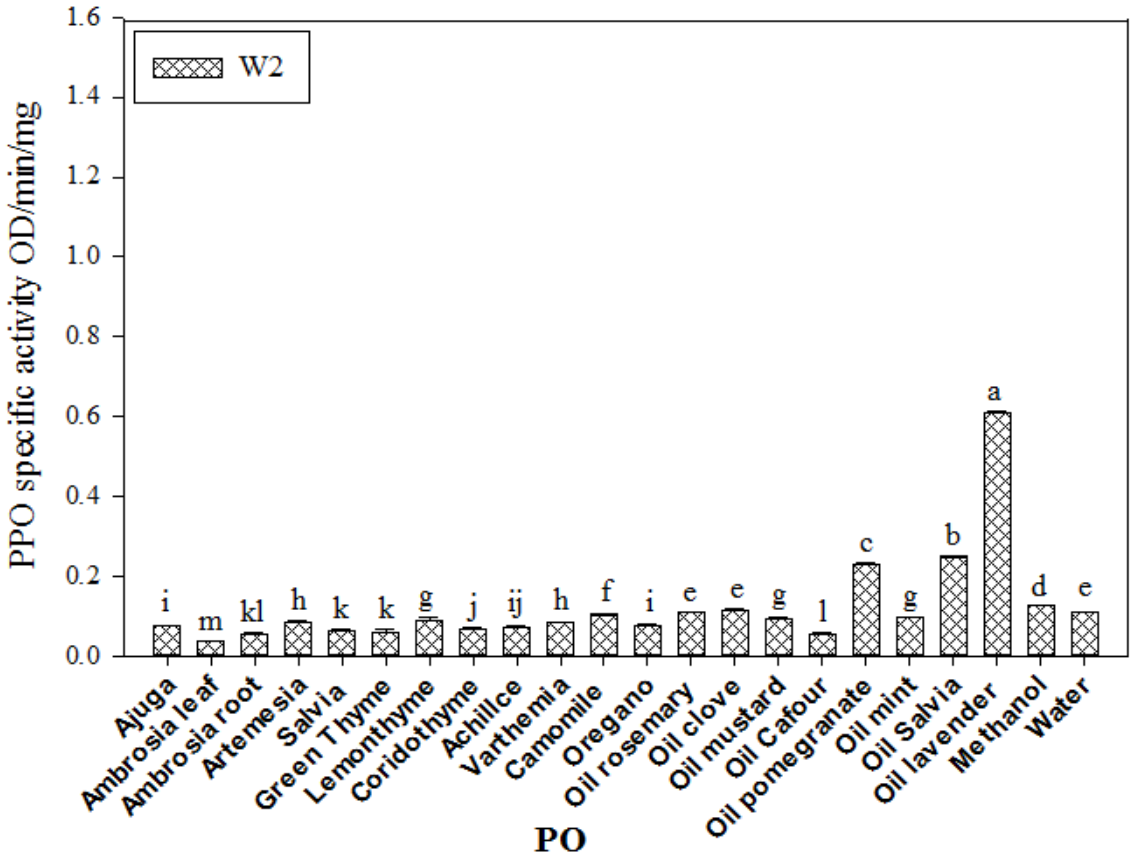

Figure 14. PPO specific activity in W2 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

In W3 cultivar PPO activity (Figure 15) reported a significant increment impact on plants that treated with oil Lavender, oil Clove, oil Pomegranate and oil Mint with readings of 0.44, 0.21, 0.19 and 0.13 OD/min/mg, respectively. Whilst, oil Salvia and Salvia extract readings of 0.111 and 0.109 OD/min/mg had no significant difference compared with control (0.111 OD/min/mg). Moreover, the rest of PO had significant decreasing impact on PPO level ranged between 4.44- 1.06 times less than control read. Here, there was no significant impact of methanol treatment compared with control.

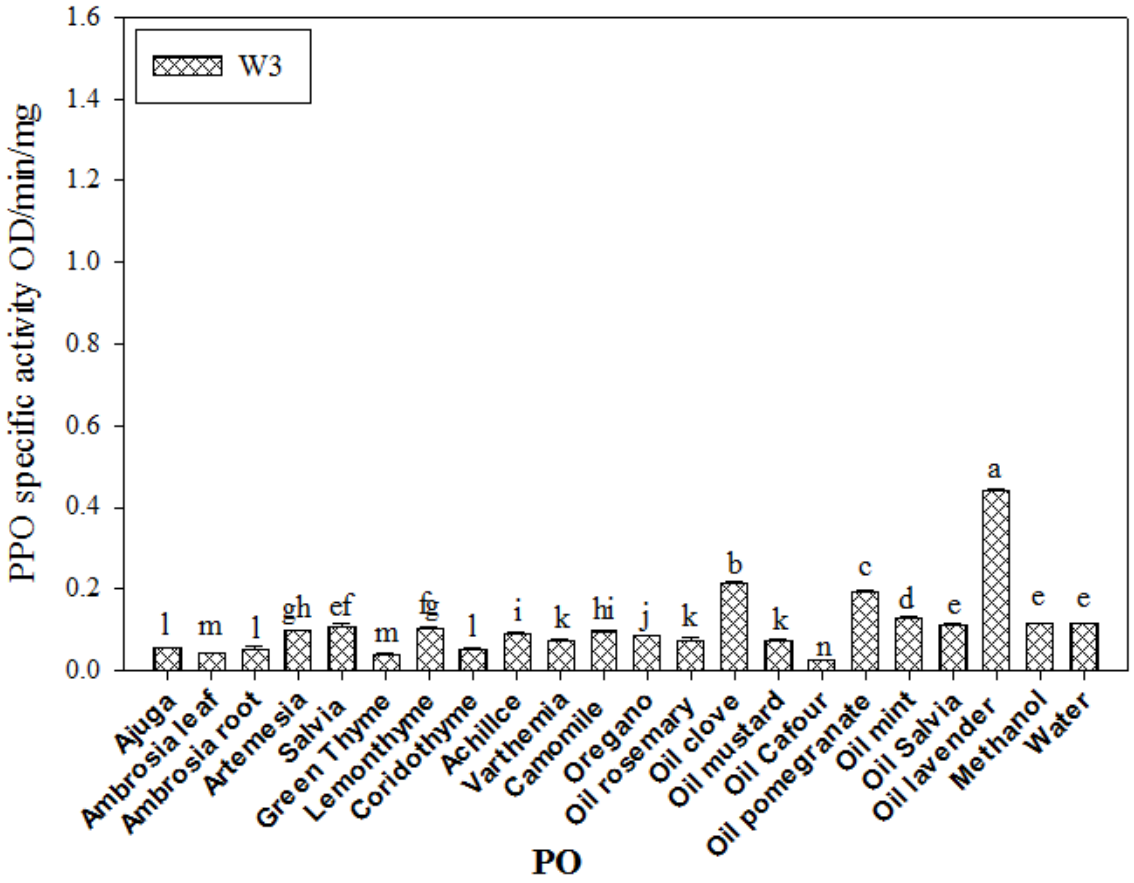

Figure 15. PPO specific activity in W3 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

W4 cultivar PPO activity results (Figure 16) showed that oil Lavender, oil Clove and oil Mustared treated plants exhibited significant increasing effect on PPO activity by 2.5, 2.4, 1.2 times, respectively more than the control (0.107 OD/min.mg). Oil Rosemary had similar impact on PPO activity after treatment compared to the control. Whilst, rest PO had significant decreasing effect on PPO activity with readings ranged between 0.099- 0.031 OD/min/mg. Methanol treatment plants were exhibited significant increasing effect of 0.118 OD/min.mg compared with control.

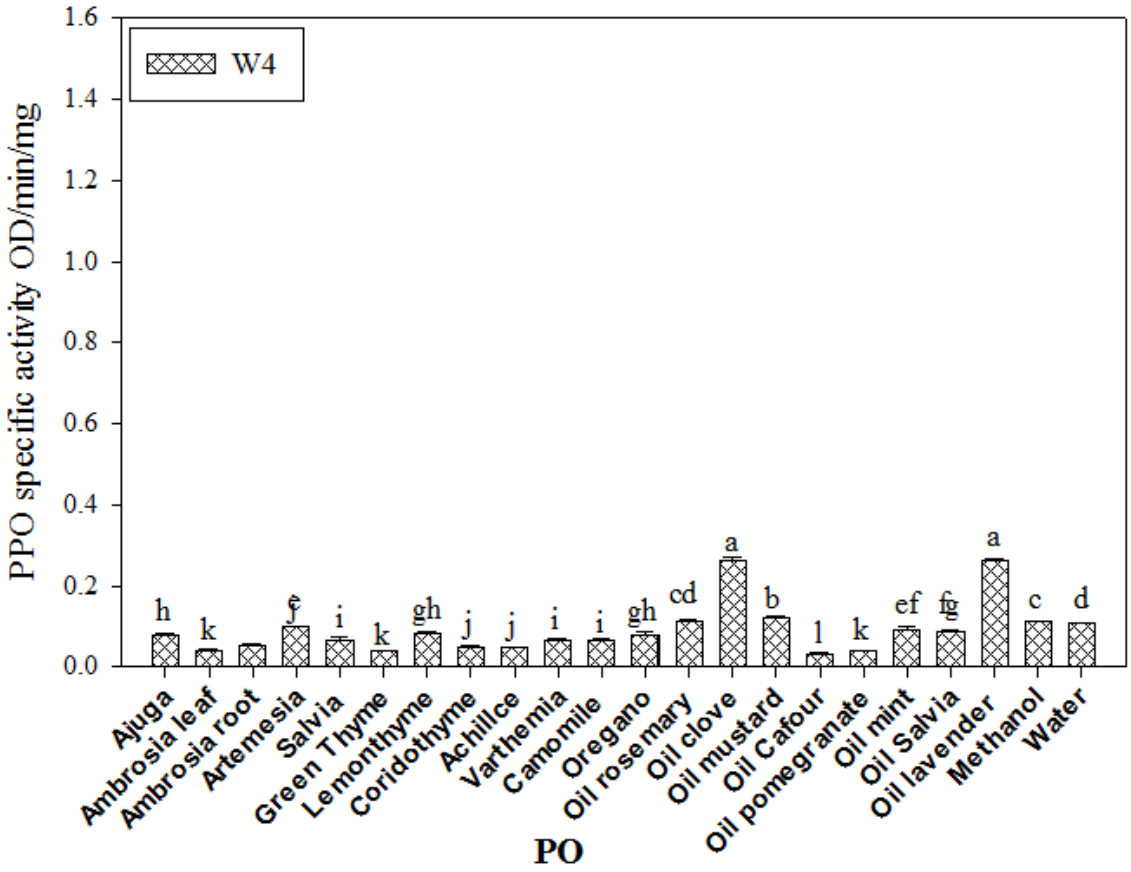

Figure 16. PPO specific activity in W4 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

On the other hand, PPO activity results for the fourth barley cultivars generally had increasing impact when it compared with control at (p= 0.05).

Figure 17 represent the PPO activity for B1 cultivar after PO treatment. Achillce, Varthemia, oil Rosemary, Camomile, Salvia, Coridothyme, oil Pomegranate, Artemesia, Green thyme, Ajuga, oil Lavender, oil Clove, Oregano, Ambrosia leaf, Lemonthyme, oil Salvia, oil Mustard and oil Cafour had significant increasing impact on PPO activity with readings of 0.465, 0.414, 0.406, 0.386, 0.351, 0.317, 0.307, 0.278, 0.241, 0.215, 0.205, 0.195, 0.127, 0.112, 0.109, 0.107 and 0.092 OD/min/mg, respectively compared

with control. Whilst Ambrosia root, oil Mint had no impact compared to control. Methanol PPO activity reading had slightly increment of 0.100 OD/min/mg compared to the control (0.071 OD/min/mg).

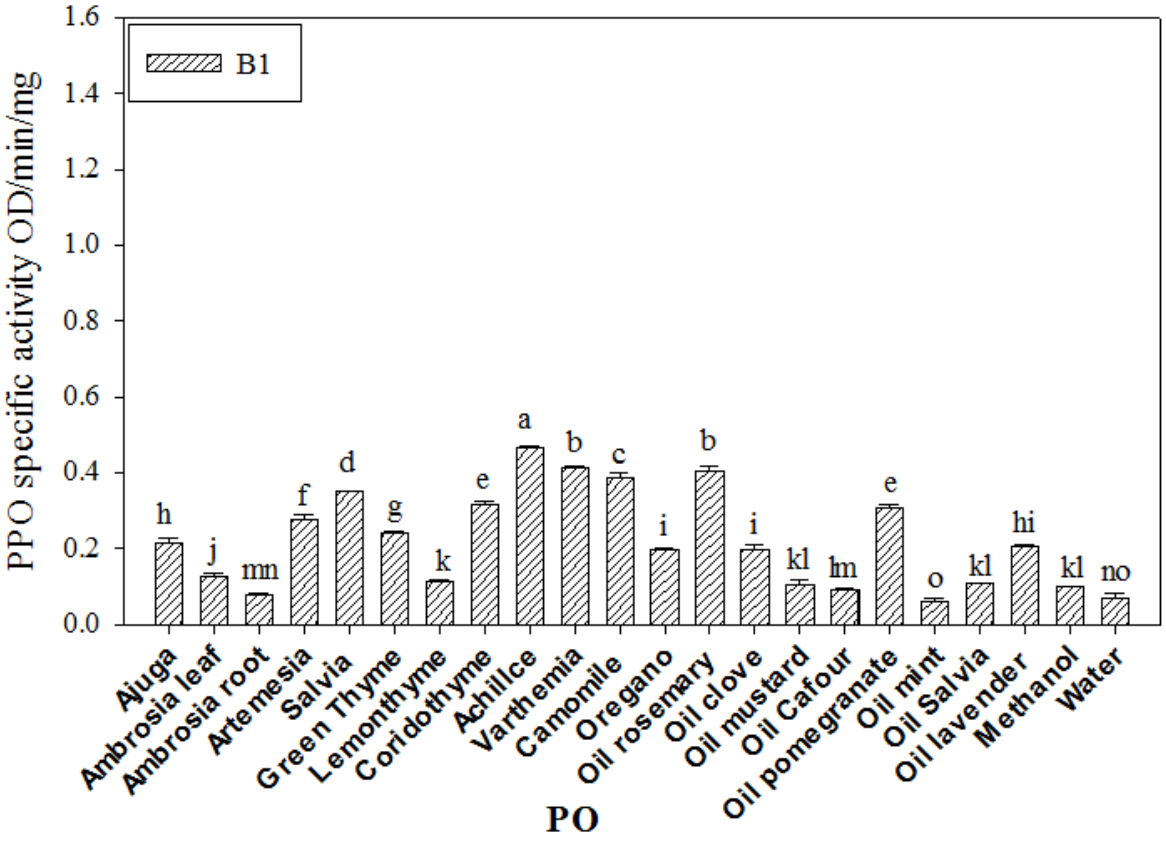

Figure 17. PPO specific activity in B1 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

B2 cultivar plants PPO activity results (Figure 18) Oil Pomegranate treatments recorded the highest reading of 0.663 OD/min.mg which is more than control by 4.9 times. Followed by, Varthemia, Achillce, Camomile, Oregano, Green thyme, Artemesia, Ajuga, Lemonthyme, Ambrosia leaf, Salvia, oil Cafour and oil Mint having readings of 0.563, 0.393, 0.367, 0.363, 0.354, 0.277, 0.221, 0.219, 0.201, 0.199, 0.163 and 0.162 OD/min/mg, respectively. While, Coridothyme, oil Lavender, oil Salvia and Ambrosia

root had similar impact on PPO activity as the control. For oil Musterd, oil Clove and oil Rosemary were had decreasing effect on PPO level by1.23, 1.34 and 1.93 times less than control level. Methanol treated leaves recorded a 0.109 OD/min/mg PPO activity that is significantly lower than control read (0.135 OD/min/mg).

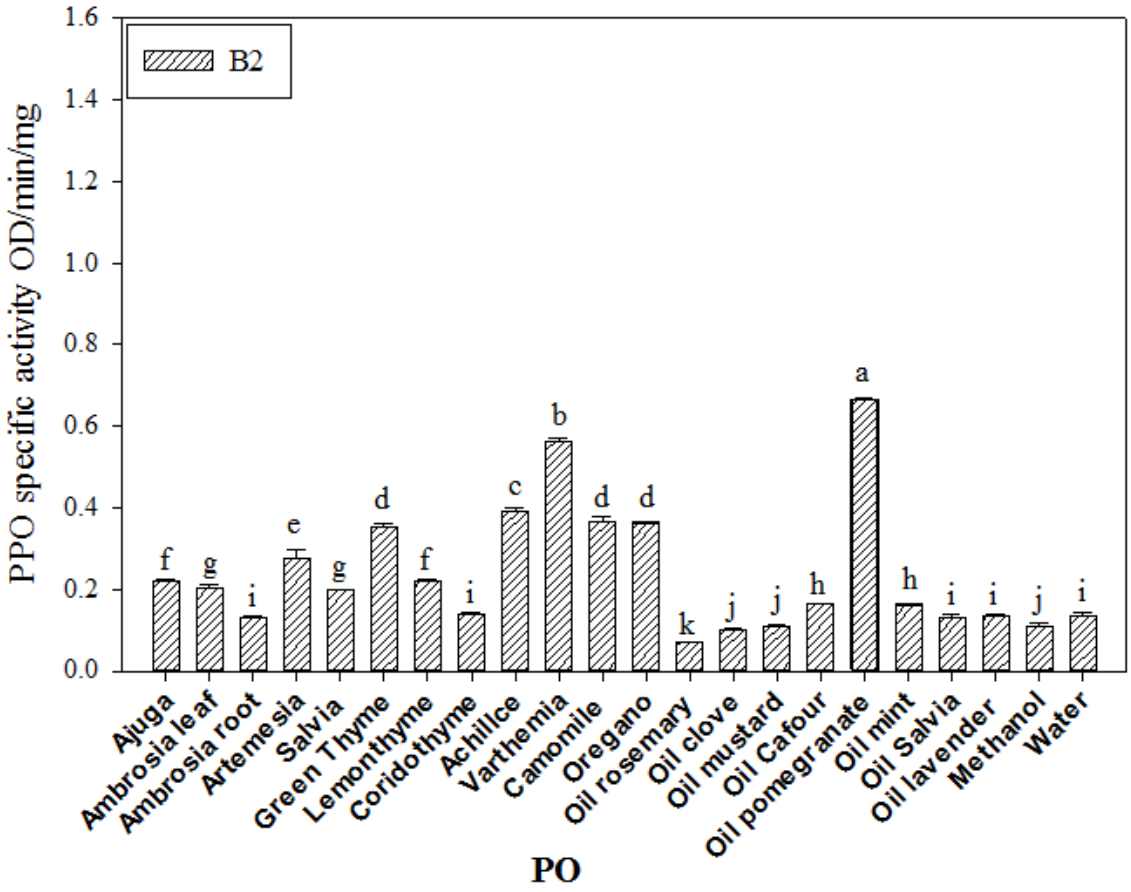

Figure 18. PPO specific activity in B2 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

PPO activity results in B3 (Figure 19) Where plants treated with Artemeasia extracts recorded the highest value (0.759 OD/min/mg), followed by Varthemia, Achillce, Camomile, Green thyme, Coridothyme, oil Clove, oil

Cafour, Ajuga, Lemon thyme, oil Rosemary, Oregano, oil Lavender, Oil mint, Ambrosia root, oil Mustard, Salvia, Ambrosia leaf and oil Pomegranate with readings ranged from 0.648 to 0.078 OD/min/mg. While, oil Salvia had no significant effect compared to control (0.071 OD/min/mg). Methanol effect showed the same effect of control.

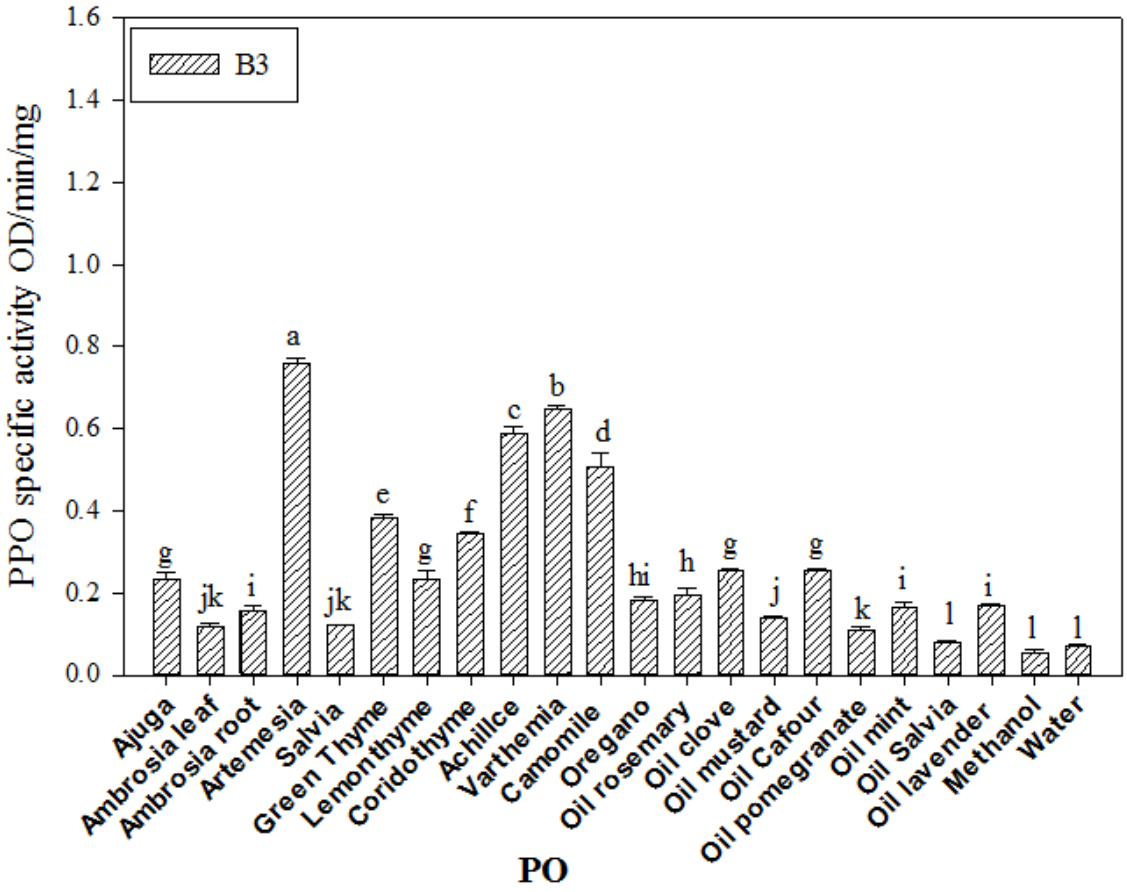

Figure 1. PPO specific activity in B3 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05

PPO activity results for B4 cultivar treated plants are shown in Figure 20. Data indicate that Oregano and Achillce treated plants increased PPO values around 8.8 and 8.4 times, respectively over control value (0.064 OD/min/mg). Followed by Camomile, Coridothyme, Varthemia, oil Clove,

oil Rosemary, Ambrosia root, Ajuga, oil Cafour, Ambrosia leaf, oil Pomegranate, oil Lavender, oil Mustard, oil Mint, Lemonthyme and Green thyme which increased PPO values significantly by 5.28, 5.04, 4.30,4.27, 4.05, 3.65, 3.53, 2.61, 2.49,, 2.28, 2.23, 2.14, 1.99 and 1.89 times, respectively over their control value. In contrast, Artemasia had decreased PPO activity to 0.025 OD/min/mg compared with control. Whilst, Salvia and oil Salvia had the same effect of control plants (0.064 OD/min/mg). Methanol had significantly higher PPO value of 0.10OD/min/mg compared with control.

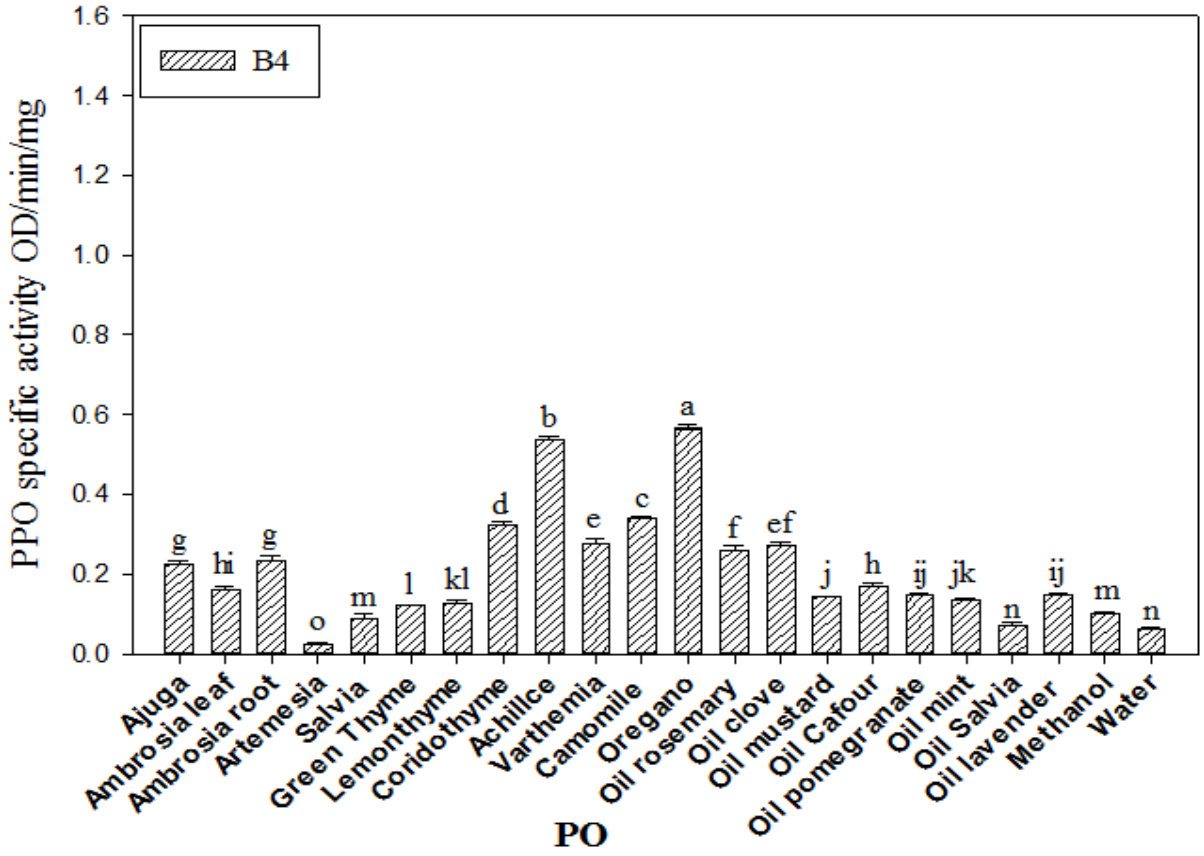

Figure 20. PPO specific activity in B4 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

2. PO treatment impact on POX activity of wheat and barley cultivars leaves

POX activity readings generally had slightly significant decreasing effect for wheat cultivars and large decreasing effect for barley after being treated with certain PO. Where, other PO had the same effect of control, at (p=0.05). Figure 21 shows POX activity readings for W1 cultivar. Where, Oregano, oil Salvia, oil Clove and oil Pomegranate had significant increasing impact on POX activity with readings of 1.248, 1,234, 1.229 and 1.211 OD/min/mg , respectively, compared with control value (1.201 OD/min/mg). In contrast, other PO exhibited slightly decreasing in POX value which ranged between 1.192- 0.978 OD/min/mg except oil Rosemary, Cammomile and Varthemia that had similar effect as the control. Whilst, methanol impact on POX activity was significantly increased compared with the control.

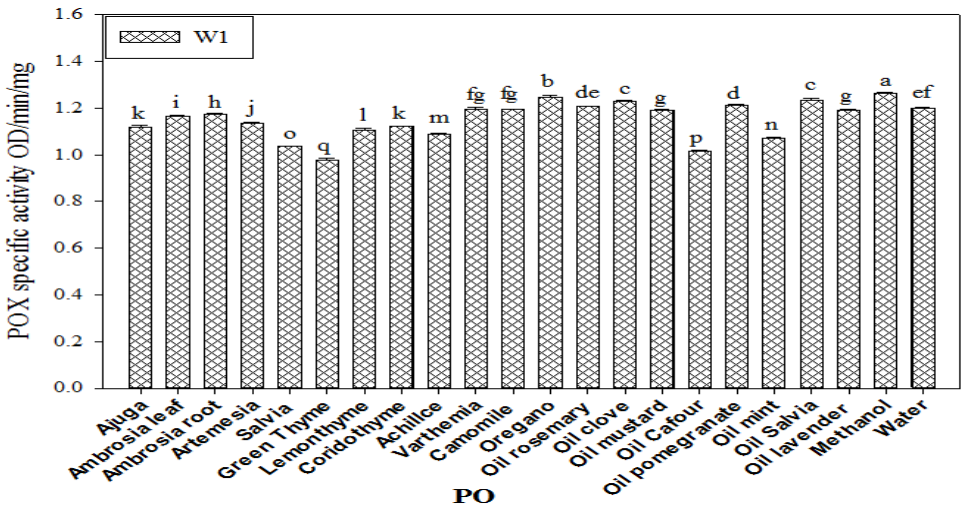

Figure 21. POX specific activity in W1 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

In W2 cultivars (Figure 22), results represent that all PO had a slightly significant decreasing effect on POX activity compared with their control values. POX activity values ranged between 0.942- 1.278 OD/min/mg. while methanol readings had similar impact to control.

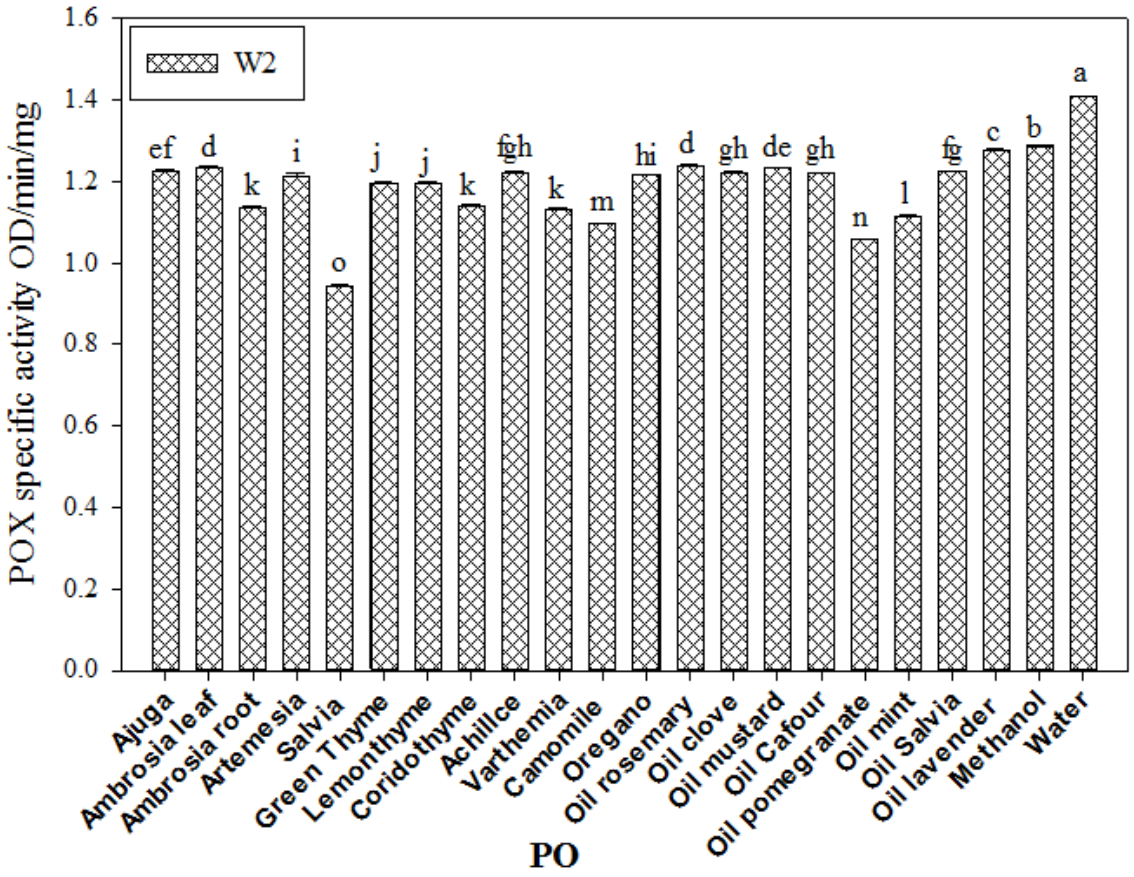

Figure 22. POX specific activity in W2 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

In W3 cultivars (Figure 23) results represent that all PO had a slightly significant decreasing effect on POX activity compared with their control

values. POX activity values ranged between 1.09- 1.24 OD/min/mg. While methanol readings had significantly decreasing effect compared to control.

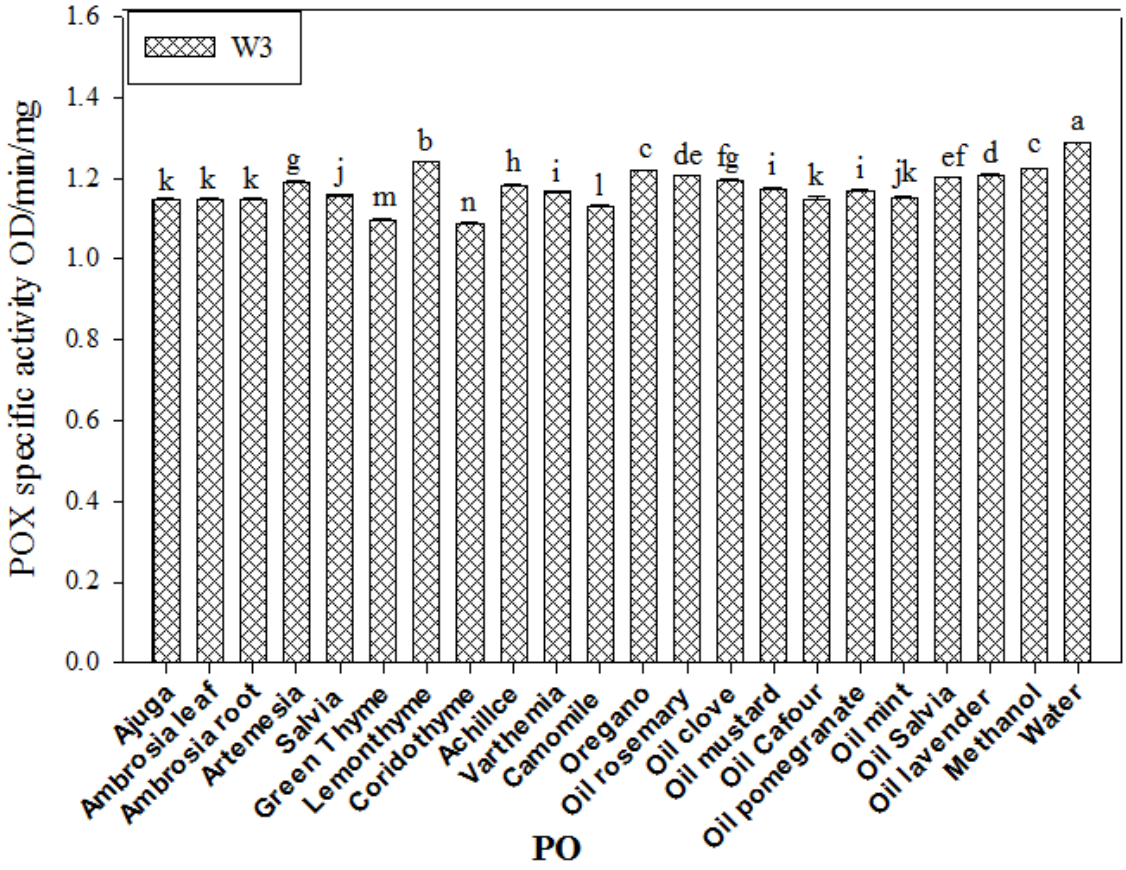

Figure 23. POX specific activity in W3 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

In W4 cultivars (Figure 24) results represent that all PO had a slightly significant decreasing effect on POX activity compared with their control values. POX activity values ranged between 0.85- 1.25 OD/min/mg. While methanol readings had significantly decreasing effect according to controls.

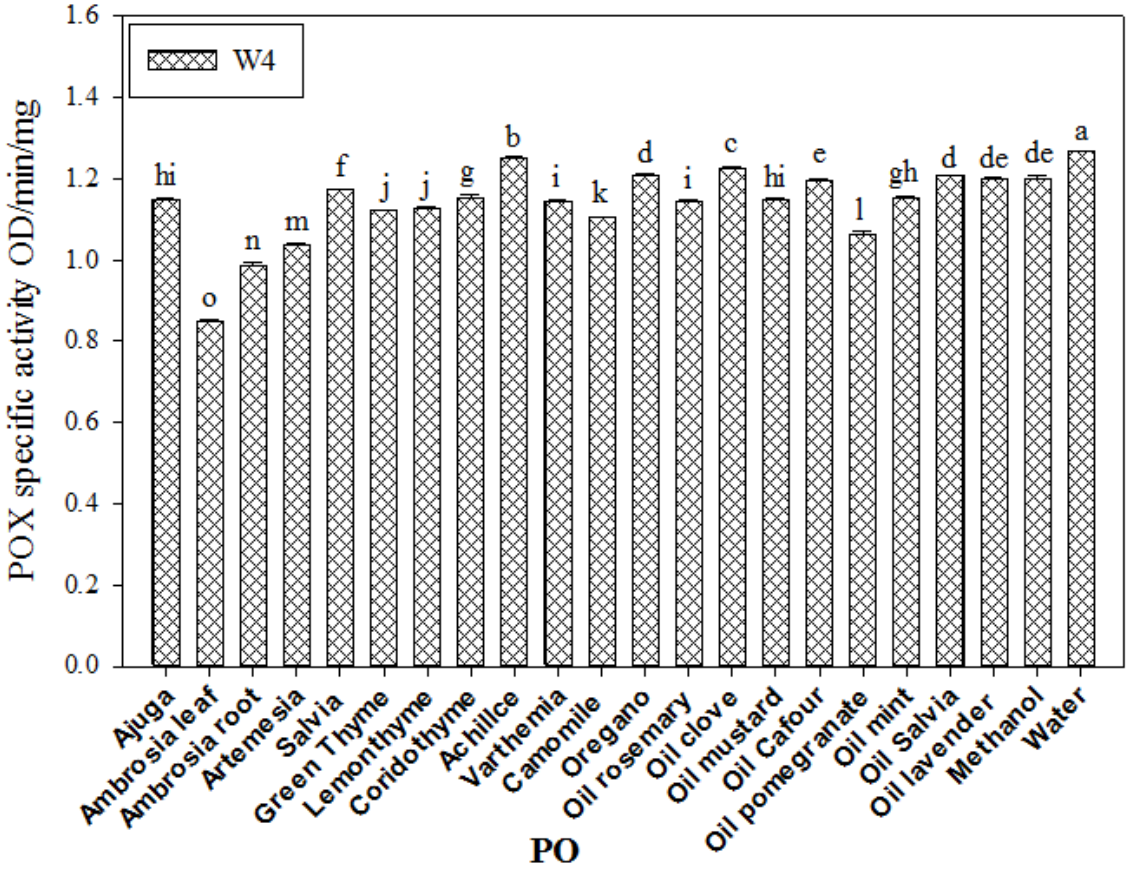

Figure 24. POX specific activity in W4 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

On the other hand, POX activity patterns in barley cultivars after PO treatments are similar to a great extent. While, in B1 cultivar (Figure 25) POX specific activity of all PO except oil Pomegranate, oil Mint, oil Lavender and oil Salvia had a magnificent decrease in POX values ranging between 0.313- 0.018OD/min/mg compared with control value (0.97 OD/min/mg).Whilst, oil Pomegranate, oil mint and oil lavender were had a significant higher POX specific activity values of 1.25, 1.19 and 1. 12 OD/min/mg, respectively compared to control. But oil Salvia effect had

slightly significant decreased value (0.99 OD/min/mg). Readings related to methanol treatment impact on POX specific activity had significantly lower value than control.

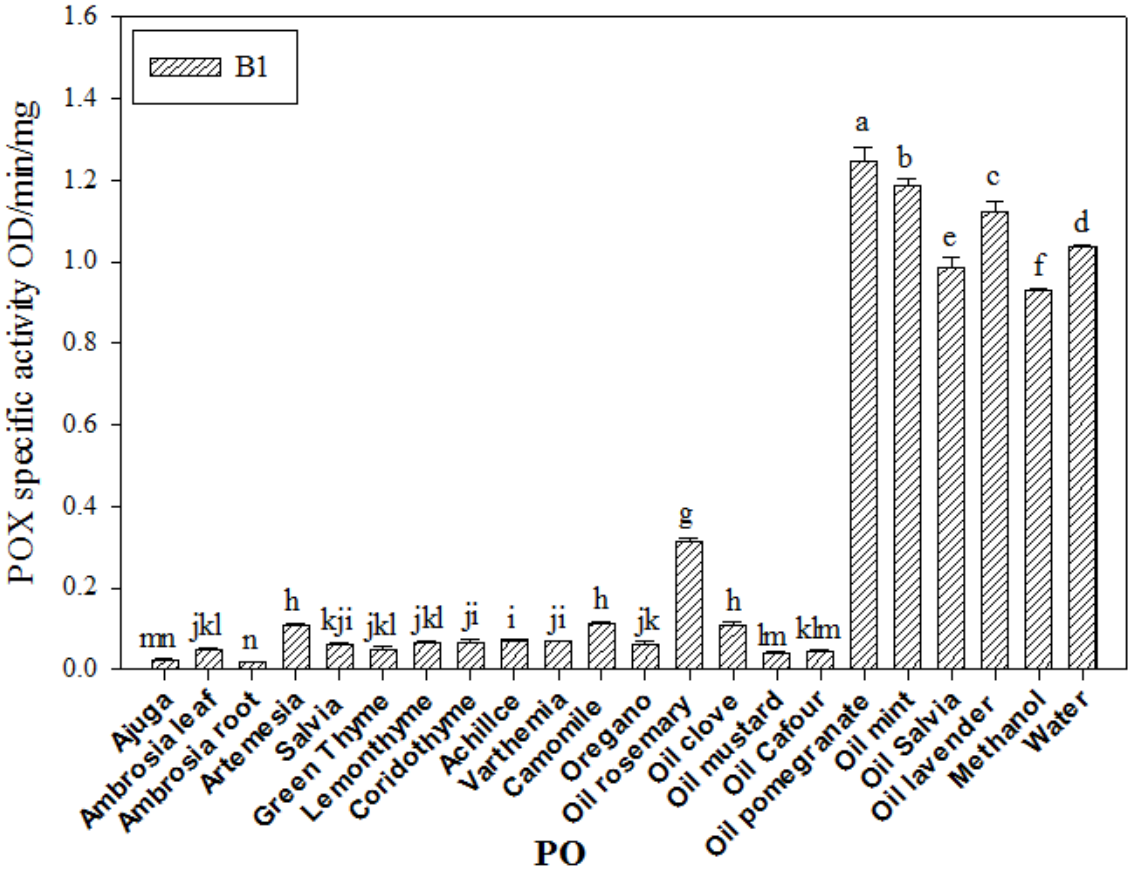

Figure 25. POX specific activity in B1 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

In B2 cultivar (Figure 26) POX specific activity of all PO except oil Pomegranate, oil Mint, oil Lavender and oil Salvia had a magnificent decrease in POX values ranging between 0.107- 0.004 OD/min/mg for B2, compared with control value (1.18 OD/min/mg). In B2 cultivar oil Pomegranate had recorded slightly higher significant value than control by

1.1 times. While, oil Mint, oil Salvia and oil lavender readings of 1.29, 1.26 and 1.25 OD/min/mg, respectively reflecting slightly decreasing effect on POX activity compared with control. Readings related to methanol treatment impact on POX specific activity had significantly lower value than control.

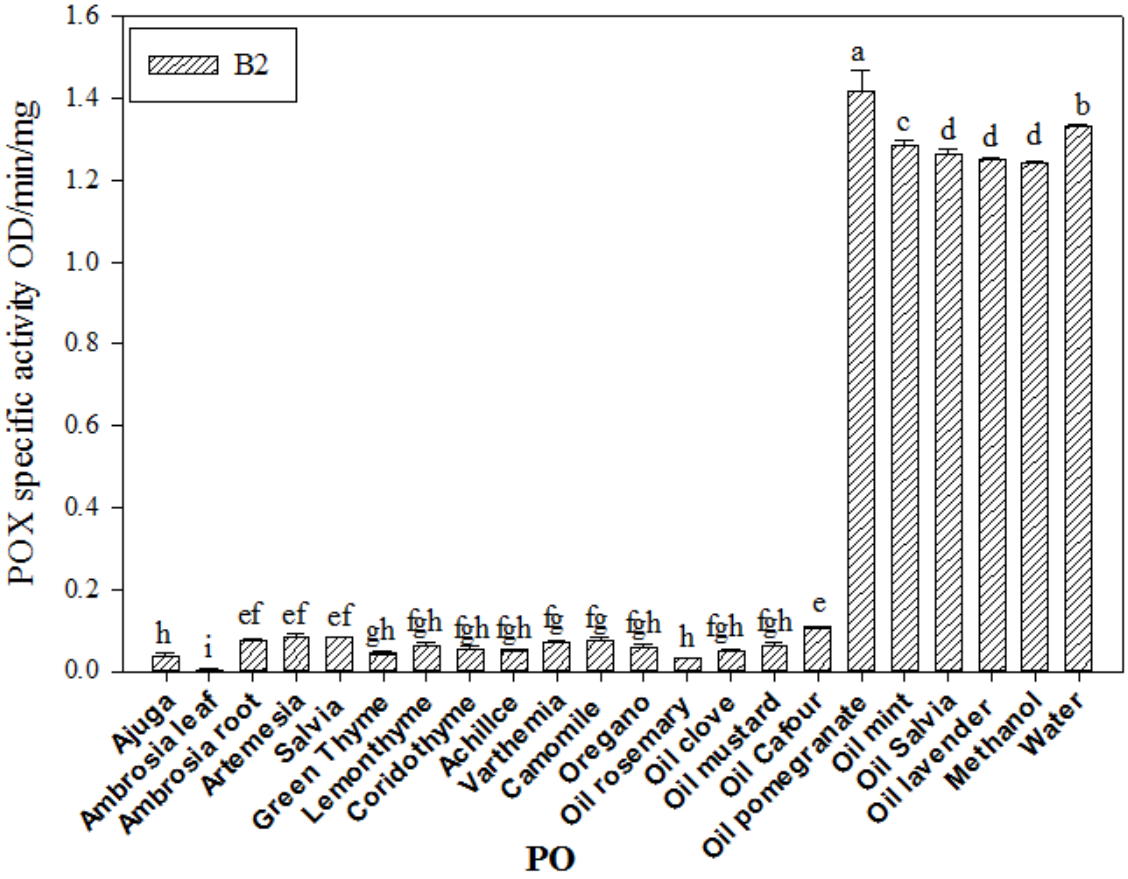

Figure 26. POX specific activity in B2 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

B3 cultivar (Figure 27) POX specific activity of all PO except oil Pomegranate, oil Mint, oil Lavender and oil Salvia had a magnificent decrease in POX values ranging between 1.004- 0.025 OD/min/mg for B3 compared with control value (1.28 OD/min/mg). In B3 cultivar oil lavender

had recorded slightly significant higher value than control by 1 time. While, oil Salvia, oil Mint and oil Pomegranate readings of 1.28, 1.19 and 1.00 OD/min/mg, respectively exhibited slightly decreasing impact on POX activity. Readings related to methanol treatment impact on POX specific activity had significantly lower value than control.

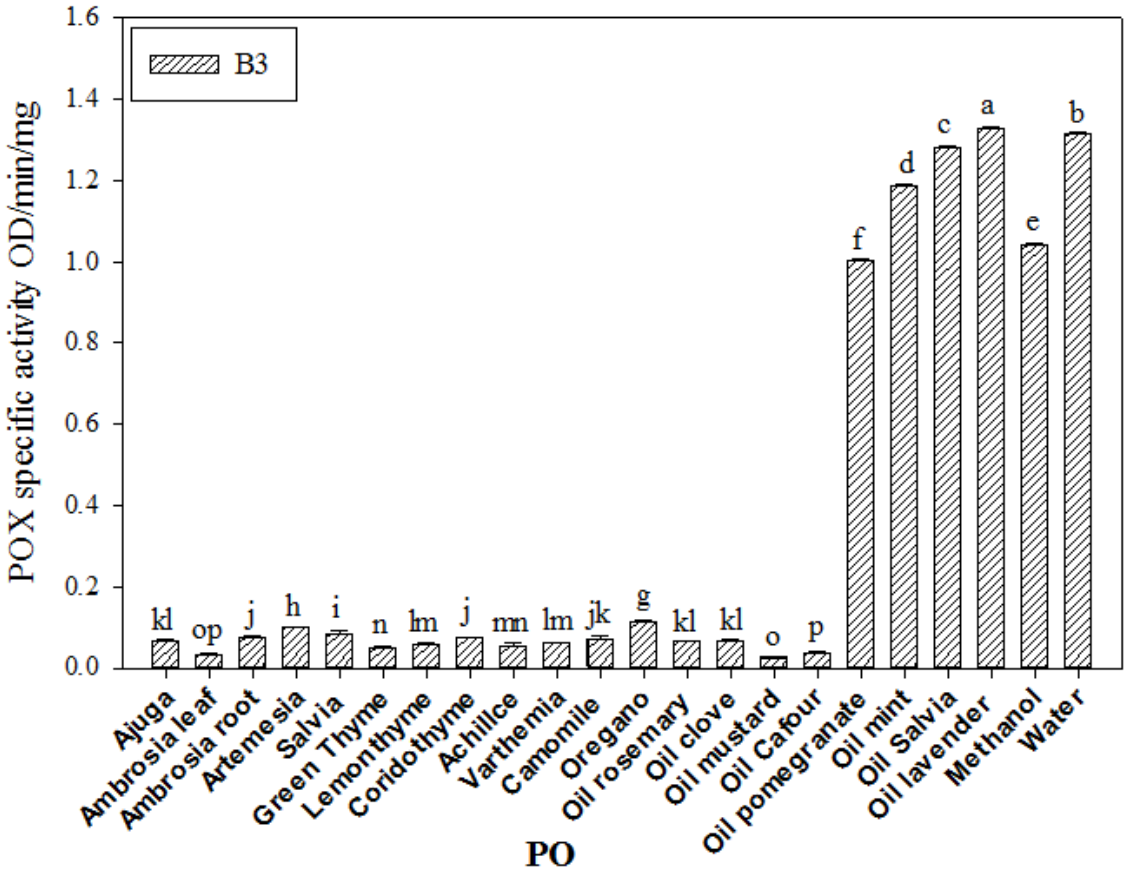

Figure 27. POX specific activity in B3 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

B4 cultivar (Figure 28) POX specific activity of all PO except oil Pomegranate, oil Mint, oil Lavender and oil Salvia had a magnificent decrease in POX values ranging between 0.118- 0.026 OD/min/mg for B4 compared with control value (0.98 OD/min/mg). B4 plants treated with, oil

Mint, oil Salvia, oil Pomegranate and oil lavender POX activity readings had slightly higher significant readings than their controls activity by 1.2, 1.1, 1.1 and 1.1 times respectively. Readings related to methanol treatment impact on POX specific activity a similar impact when compared with control.

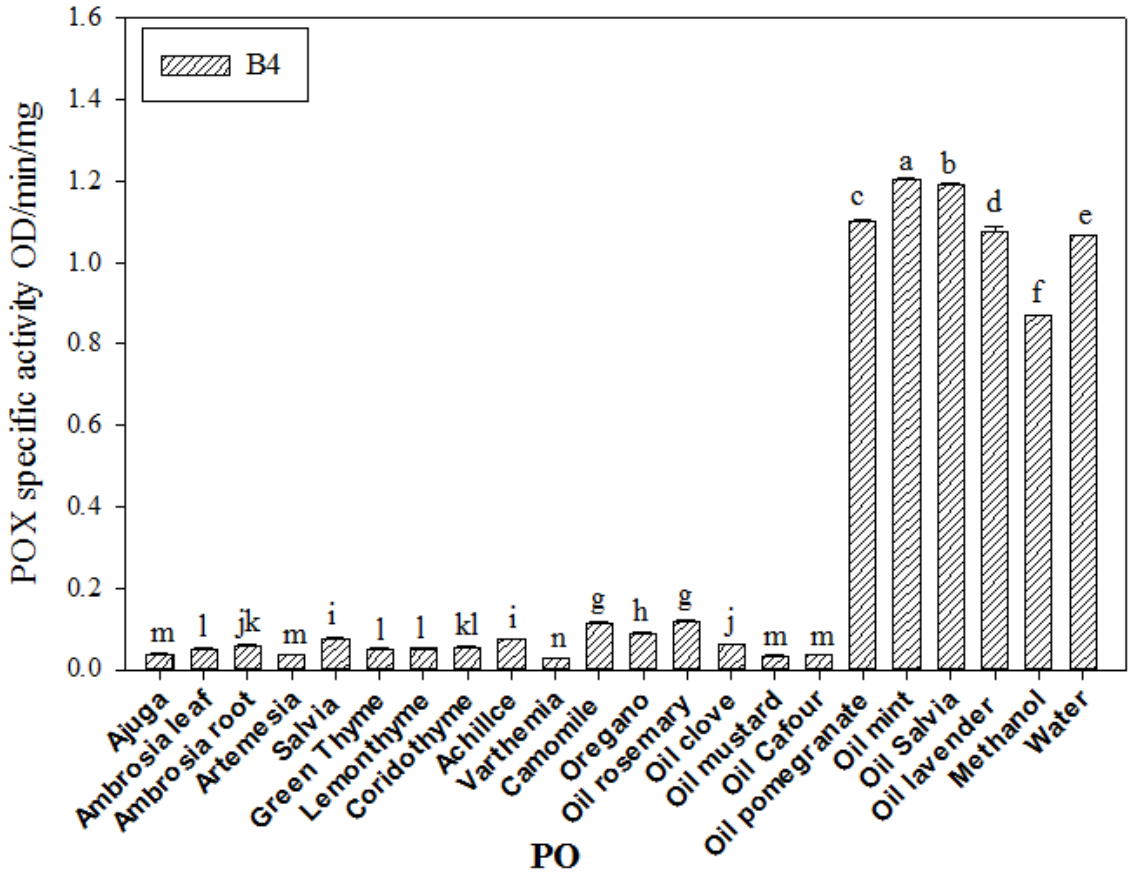

Figure 28. POX specific activity in B4 cultivar leaves at 3 foliar stage after PO treatment, results represent mean and Std of 3 replicate. Similar latter represent same effect at P= 0.05.

5 Discussion

Isolated *Ustilago* sp. fungi from infected samples were successfully identified and verified by both morphological and molecular tools. Furthermore, the set of 26S primers were successful in answering that isolated fungi belong to *Ustilago* sp. according to (Basse et al., 2000).

Potential antifungal activity of the medicinal PO against *Ustilago* sp. fungi in-vitro carried out in this study showed that 70% of PO used have some antifungal activity. Fungal growth inhibition percentage ranged between 33-60%. Results are in accordance with several studies. Hence leaves extract of *poisonous phanergamic*, *Nerium odorum*, senna and Indian mallow is used in controlling wheat rust fungi, different plant extracts such as: *Azardiachta indica*, *Artemessia annua*, *Eucalyptus globulus*, *Ocimum sanctum* and *Rheum emodi* were tested to control brinjal wilt pathogen (Ayoub & Niazi, 2001; Dey et al., 2013; Joseph et al., 2008; Rahber-Bhatti, 1988). In which, it reported that plant extracts are effective biocontrol agents against different plant pathogen, this could be to toxic components to pathogen (Shabana et al., 2017). These active components are varied according to plant species, age, plant tissues, environmental factors and extraction solvents (Demo & Oliva, 2008; Pinelo, Manzocco, Nunez, & Nicoli, 2004; Webster, Taschereau, Belland, Sand, & Rennie, 2008). These factors could only be determined by extensive studies. In addition, the season of harvested plant materials and the growing site of it may have a role in plant material

composition and antifungal behavior as a result (Putievsky, Ravid, & Dudai, 1986).

Host plants use two ways to suppress diseases and defend themselves against pathogen attack and overcomes stress factors. Direct blocking of the pathogen proliferation and/ or indirect induction of plant resistance pathways (Shabana et al., 2017).

Plants in general can promote induce resistance in response to pathogen attack, insect, rhizomicrobes or by treatment with chemical or physical agents (Pieterse et al., 2014). Studies of different plant- pathogen systems have shown that plants can activate at least six defense signaling pathways involving different regulators depending on the types of infection or related situations (Ton, Van Pelt, Van Loon, & Pieterse, 2002; Walling, 2000).

Resistance induction in plants was associated by variable antimicrobial compounds such as oxido reductive enzymes like POX, PPO and others (Chittoor, Leach, & White, 1999; Kolattukudy, Mohan, Bajar, & Sherf, 1992). However the possible functions of peroxidases in plant resistant mechanisms remain unclear (Datta & Muthukrishnan, 1999). But several studies are documented the possible function that refer to POX and PPO. Oxidative enzymes have been shown to be participation in oxidize phenolics and other metabolites and it play a key role in lignification of plant cells among defense mechanisms, also it have the ability for H_2O_2 degradation in

plant cells (Dean & Ku-ç, 1987, Svalheim & Robertsen, 1990; Van Loon, 1997).

Several researches documented that changes in peroxidase activity was demonstrated in many hosts such as wheat (Flott, Moerschbacher, & Reisener, 1989) and barley (Kerby & Somerville, 1989, 1992) as a response of plant induced resistance. These indicator enzymes have been associated in different crops after pathogen infection interactions such as cabbage infected with *Fusarium oxysporum*, onion infected with *Botrytis*, sunflower infected with *Sclerotinia sclerotiorum*, soybean infected with *Phytophthorameg asperma* and bean infected with *Rhizoctonia* (Goodman, Király, & Wood, 1986; Datta & Muthukrishnan, 1999).

Moreover, many workers documented inducing of pathogen related enzymes and others followed application of chemicals that mimic pathogen infection effect or wound responses (Fu & Dong, 2013; Pieterse et al., 2014). Similar results have previously been reported in apple, pea, cabbage, tomato and lettuce post b- Aminobutyric acid (BABA) treatment (Barilli, Prats, & Rubiales, 2010; Cohen, Rubin, & Vaknin, 2011; Quaglia, Ederli, Pasqualini, & Zazzerini, 2011; Sharma, Bruns, Butz, & Finckh, 2012; van der Wolf et al., 2012), also in elicitors- plant interactions experiments, since such a study found that Acibenzolar-S-methyl (ASM) applied to mango was accompanied by increased gene expression of POX and PPO (Lin, Gong, Zhu, Zhang, & Zhang, 2011). Same result was obtained in case of muskmelons treatments

(Zhang, Wang, Deng, Xie, & Wang, 2011) and others. Other results were reported in very rare studies that documented decreasing of POX activity in *Brassica napus* following SA treatment and nitric oxide elicitors (Kazemi, Khavari-Nejad, Fahimi, Saadatmand, & Nejad-Sattari, 2010). Others found that treated wheat heads with SA elicitors had not induces POX genes in contrast with MJ (Mohammadi & Kazemi, 2002).

Changes in PPO and POX level in this study could be attributed to different likely reasons, such as host genotype and the growth stage of plants, type and character of elicitor used and resistance pathway cross-talking. In which, the expression of induced resistance is host genotype dependent according to (Dann, Diers, Byrum, & Hammerschmidt, 1998; Hijwegen & Verhaar, 1995; Martinelli, Brown, & Wolfe, 1993; Resende et al., 2002; Tucci, Ruocco, De Masi, De Palma, & Lorito, 2011). For example Walters, Havis, Paterson, Taylor, & Walsh, (2011) found that expression of induced resistance in spring barley cultivars are varied significantly following treatment with a combination of BABA, ASM and cis jasmine, furthermore some cultivars did not express induced resistance at all. Similar results are obtained in tomato cultivars after treated with BABA chemical elicitor (Sharma, Butz, & Finckh, 2010). Although, several studies documented that plant extracts and oils have a crucial role in plant disease management, the mechanisms of plant protection by these elicitors are still un understood, (Ayoub & Niazi, 2001; Dey et al. 2013; Elsharkawy & El-Sawy, 2015;

Joseph et al., 2008; Sowjanya & Chary, 2012). However, participation of induced resistance was reported (Fokkema, 1993).

It could be concluded that the elicitor character may play a crucial role in efficacy performance of inducing resistance in plants. Data obtained agreed with a previous study which documented that Butanediol and a new organic compound (PC1) were had differences in their capability to activate induce resistance in the same host plant (Cortes-Barco, Hsiang, & Goodwin, 2010). These findings suggest that elicitor may determine which genes or pathways will be activated. Plants generally reported to have over 4 million type of chemical molecules, including10 thousand as secondary metabolites that play part in plant defensive (Wilcoxson, 1996). It is worth to point out that PO composed of different materials and molecules and there is no clear information about the quantity and quality of induced defense molecules in PO present. Hence, it may present in different concentration and might be more than one induced defense molecules per each PO used in this study. This is come in agreement with report documenting that not only elicitor type affect expression of induced resistance, but also the dose of elicitors applied is affected significantly (Boughton et al., 2006). Indeed, oxidative enzymes are transcribed from multigene families as reported by Almagro et al.,(2008). Moreover, single plant species may have up to 40 different isoforms according to Dickinson (2004). Also, others were reported that regulatory

mechanisms and pathways among PPO and POX are different and it depend on the interaction between elicitors and host plants (Almagro et al., 2008; Seo, Lee, Xiang, & Park, 2008; Ton et al., 2002). Novel host proteins were found only conditionally and in some cases remain poorly characterized (Datta & Muthukrishnan, 1999). This suggest that plants express proteins according to its life history.

Host plants compromise their resources and energy to investigate balance between growth and defense through cross-talking between their hormones and resources, it try to protect itself from biotech or a-biotech danger at the expense of their growth and development (Fu & Dong, 2013).

Different articles documented not only interfering but also antagonism between plant defense mechanisms such as SAR and ISR mechanisms, which they are SA dependent and JA acid dependent pathways respectively (Datta & Muthukrishnan, 1999; Zeller, 2006). Several reports documented that interaction between pathogen and insect resistance not only as negative cross-talk (Thaler, Humphrey, & Whiteman, 2012), but it could be find no effect (Ajlan & Potter, 1992; Inbar, Doostdar, Sonoda, Leibee, & Mayer, 1998), however others reported positive effect (Hatcher & Paul, 2000; Stout, Fidantsef, Duffey, & Bostock, 1999).

It could be suggested that changes in POX and PPO expression level also might be due to presence of basal resistance in plants (Co'rdova-Campos

Adame-Álvarez, Acosta-Gallegos, & Heil, 2012). However, not all host pathogen interaction respond to elicitors in the same way, the findings in this aspect are rare and not distinct but involving of genotype dependency was reported (Herman & Williams, 2012). In addition to all previous explanation, it could be that induction of oxidative enzymes was delayed or not induced during the studying period time (Datta & Muthukrishnan, 1999).

6 Conclusion

Based on the findings of this study, in-vitro antifungal activity of different Palestinian herbal plant extracts and local commercial oils, showed that the extract components are effective against fungal growth such as Coridothyme, Salvia, Varthemia.

Moreover, applying PO on wheat and barley cultivars showed varied induced responses according to the level of POX and PPO in each cultivar. For example, Oregano, Clove or Lavender and Pomegranate, Achillce or Cammomile oil have effective induction for resistance indicator enzymes in wheat and barley, respectively.

It could be concluded that using PO as plant resistance elicitor is promising for the loose smut disease management in barley and wheat. Also, it can be considered as a new, safe and risk free biocontrol agents for plant diseases management and also, producing more crops with less synthetic chemicals.

7 Recommendation

This study is highly recommended that using of PO could be induced plant resistance. In fact, many factors affect plant defensive mechanisms, even though further studies is recommended to determine and isolate the chemical defensive molecules have the potential for pathogen growth inhibition and induce plant resistance mechanisms. Further study with wheat and barley should investigate the resistance induction phenomenon at other growth stages and different elicitors concentrations. Moreover studying of phytotoxicity and the efficacy of candidate PO on growth parameters of treated crops is recommended. While time and budget limitation prevent conducted this experiment. Although PPO and POX enzymes are the most common type plant PR proteins analyzed as indicator of resistance induction but assessment of other PR are recommended.

8 Abstract in Arabic (الملخص)

تحفيز مقاومة النبات طبيعيا بواسطة مستخلصات نباتية فلسطينية لمكافحة مرض التفحم المكشوف في محصولي القمح والشعير

الطالبة: بشرى كرسوع

المشرفة: د. رنا سمارة

الملخص

يعد محصـــولي القمح والشـــعير من أهم المحاصـــيل الزراعية الُمنتجة بالعالم، ولكن يهددها خطر الاصابة بالأمراض النباتية حيث أنها تعتبر عائلا للعديد من الممرضات النباتية والتي تسبب الكثير من الخســائر الاقتصــادية في هذه المحاصــيل. احدى هذه الأمراض الشــائعة التي تصــيب هذه المحاصــيل في فلســطين هو مرض التفحم المكشــوف على القمح (*Ustilago tritici*) و التفحم المكشـوف على الشـعير (*Ustilago nuda*) . وتتم مكافحة هذا المرض بالعديد من الطرق منها اســـتخدام المبيدات الفطرية، والتي يتم العمل على اســـتبدالها بطرق أكثر أمناً ومواءمةً للإنتاج الزراعي المستدام والأمن الغذائي.

في هذه الدراســة تم اختبار العديد من مســتخلصــات نباتات فلســطينية برية ليس فقط ضــد الفطر المسبب لمرض التفحم المكشوف وانما أيضاً تم اختبار فعالية هذه المستخلصات في تحفيز مسارات مقاومة النباتات العائل للأمراض، وذلك من خلال قياس مســـتوى النشـــاط الإنزيمي للبروتينات المصـــاحبة للاصـــابة بالممرض (POX وPPO) والتي تعتبر كمؤشـــر لحدوث تحفيز مســـارات المقاومة للنباتات.

كانت النتائج التي تمخضت عن هذه الدراسة تبين أنَ 70% من المستخلصات النباتية المستخدمة في هذه الدراسة كان تملك فعالية ضد الفطر المسبب لمرض التفحم المكشوف، حيث أنَ الزعتر الفارسي احتل المرتبة الأولى بقيمة 61%في تثبيط نمو الفطر ،من ناحية أخرى اظهرت النتائج أن بعض المستخلصات النباتية كان لها تأثير معنوي تحفيزي لنشاط الانزيمات POX و/ أو PPO مقارنة مع العينة المرجع لكل من أصناف القمح والشعير المستخدمة، حيث أن (الأوريجانو، القرنفل و الخزامى) و (الرمان، الجعدة و البابونج) استطاعت ان تحفز نشاط الانزيمين معا في أصناف القمح و الشعيرعلى الترتيب.

هذه الدراسة تمكنت من التحقق من امتلاك المستخلصات النباتية دور مهم وفعال في تحفيز المقاومة الداخلية للنباتات. وبالتالي فهذه النتائج تفتح المجال لتقليل استخدام المبيدات الكيميائية لوقاية النباتات. هذا البحث ربما يمثل خطوة لمكافحة مرض التفحم المكشوف في محصولي القمح والشعير باستخدام مستخلصات نباتية.

كلمات مفتاحية: القمح، الشعير، التفحم المكشوف، تحفيز مقاومة النبات، المستخلصات النباتية، الانزيمات النباتية المصاحبة للمرض.

9 References

Ajlan, A. M., & Potter, D. A. (1992). Lack of effect of tobacco mosaic virus-induced systemic acquired resistance on arthropod herbivores in tobacco. *Phytopathology*, 82(6), 647-651.

Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., & Pedreno, M. A. (2008). Class III peroxidases in plant defense reactions. *Journal of Experimental Botany*, 60(2), 377-390.

Aman, M., & Rai, V. R. (2015). Antifungal activity of fungicides and plant extracts against yellow sigatoka disease causing *Mycosxaerella musicola*. *Current Research in Environmental and Applied Mycology*, 5(3), 277-284.

Ames, N. P., & Rhymer, C. R. (2008). Issues surrounding health claims for barley. *The Journal of Nutrition*, 138(6), 1237S-1243S.

Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology and Evolution*, 19(10), 535-544.

Ayoub, M., & Niazi, A. U. (2001). Control of wheat rust by leaves extract of poisonous phanergamic plants. *Journal of Biological Sciences*, 1, 490-491.

Baraka, M. A., Radwan, F. M., Shaban, W. I., & Arafat, K. H. (2011). Efficiency of some plant extracts, natural oils, biofungicides and fungicides against root rot disease of date palm. *J. Biol. Chem. Environ*. Sci, 6(2), 405-429.

Barilli, E., Prats, E., & Rubiales, D. (2010). Benzothiadiazole and BABA improve resistance to *Uromycespisi* (Pers.) Wint. In *Pisumsativum* L. with an enhancement of enzymatic activities and total phenolic content. *European Journal of Plant pathology*, 128(4), 483-493.

Basse, C. W., Stumpferl, S., & Kahmann, R. (2000). Characterization of a *Ustilago maydis* gene spesifically induced during the biotrophic phase: evidence for negative as well as positive regulation. *Molecular and cellular biology*, 20(1), 329-339.

Bengtsson, M., Wulff, E., Jørgensen, H. L., Pham, A., Lübeck, M., & Hockenhull, J. (2009). Comparative studies on the effects of a yucca extract and acibenzolar-S-methyl (ASM) on inhibition of *Venturia inaequalis* in apple leaves. *European Journal of Plant Pathology*, 124(2), 187-198.

Benhamou, N., Lafontaine, P. J., & Nicole, M. (1994). Induction of systemic resistance to Fusarium crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathology*, 84(12), 1432-1444.

Benhamou, N., Kloepper, J. W., Quadt-Hallman, A., & Tuzun, S. (1996). Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. *Plant Physiology*, 112(3), 919-929.

Boughton, A. J., Hoover, K., & Felton, G. W. (2006). Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. *Entomologia Experimentaliset Applicata*, 120(3), 175-188.

Burketova, L., Trda, L., Ott, P. G., & Valentova, O. (2015). Bio-based resistance inducers for sustainable plant protection against pathogens. *Biotechnology Advances*, 33(6), 994-1004.

Bussmann, R. W., Glenn, A., Meyer, K., Kuhlman, A., & Townesmith, A. (2010). Herbal mixtures in traditional medicine in Northern Peru. *Journal of Ethnobiology and Ethnomedicine*, 6(1), 10-21.

Calvo, P., Nelson, L., & Kloepper, J. W. (2014). Agricultural uses of plant biostimulants. *Plant and Soil*, 383(1-2), 3-41.

Chaudhary, R. F., & Chaudhari, M. G. (2013). Effect of fungicides and plant extracts on uredospores germination *of Puccinia recondita* f. sp. *tritici*. *Bioscan*, 8(1), 59-62.

Chittoor, J. M., Leach, J. E., & White, F. F. (1999). Induction of peroxidase during defense against pathogens. In: Swapan K.Datta and Subbaratnam Muthukrishnan (ED) *Pathogenesis-related proteins in plants* (pp 171-193), CABI International, Wallingford, *UK*.

Cohen, Y., Rubin, A. E., & Vaknin, M. (2011). Post infection application of DL-3-amino-butyric acid (BABA) induces multiple forms of resistance against *Bremia lactucae* in lettuce. *European Journal of Plant Pathology*, 130(1), 13-27.

Cortes-Barco, A. M., Hsiang, T., & Goodwin, P. H. (2010). Induced systemic resistance against three foliar diseases of *Agrostis stolonifera* by (2R, 3R) -butanediol or an isoparaffin mixture. *Annals of Applied Biology*, 157(2), 179-189.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.

Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343-356.

Cuthbertson, A. G. S., Blackburn, L. F., Northing, P., Luo, W., Cannon, R. J. C., & Walters, K. F. A. (2009). Leaf dipping as an environmental screening measure to test chemical efficacy against *Bemisia tabaci* on poinsettia plants. *International Journal of Environmental Science and Technology*, 6(3), 347-352.

Co'rdova-Campos, O., Adame-Álvarez, R. M., Acosta-Gallegos, J. A., & Heil, M. (2012). Domestication affected the basal and induced disease resistance in common bean (*Phaseolus vulgaris*). *European Journal of Plant Pathology*, 134(2), 367-379.

da Rocha, A. B., & Hammerschmidt, R. (2005). History and perspectives on the use of disease resistance inducers in horticultural crops. *HortTechnology*, 15(3), 518-529.

Datta, S. K., & Muthukrishnan, S. (1999). Induction of Peroxidase During Defense Against Pathogens. In: Swapan K. Datta, Subbaratnam Muthukrishnan (ED). *Pathogenesis-related proteins in plants* (pp. 176- 199). CRC press.

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(3), 271-278.

De Cal, A., Sztejnberg, A., Sabuquillo, P., & Melgarejo, P. (2009). Management Fusarium wilt on melon and watermelon by *Penicillium oxalicum*. *Biological Control*, 51(3), 480-486.

Dean, R. A., & Kuć, J. (1987). Rapid lignification in response to wounding and infection as a mechanism for induced systemic protection in cucumber. *Physiological and Molecular Plant Pathology*, 31(1), 69-81.

Deepak, S. A., Raj, S. N., Umemura, K., Kono, T., & Shetty, H. S. (2003). Cerebroside as an elicitor for induced resistance against the downy mildew pathogen in pearl millet. *Annals of Applied Biology*, 143(2), 169-173.

Deepthi, N, Madhusudhan, KN., Uday Shankar, A. C., Bhuvanendra Kumar, H., Prakash HS., & Shetty, HS. (2007). Effect of Plant Extracts and Acetone Precipitated Proteins from Six Medicinal Plants Against Tobamo virus Infection. *International Journal of Virology*, 3(2), 80-87.

Dellavalle, P. D., Cabrera, A., Alem, D., Larrañaga, P., Ferreira, F., & Dalla Rizza, M. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp.*Chilean. Journal of Agricultural Research*, 71(2), 231.

Demo, M. S., & Oliva, M. (2008). Antimicrobial activity of medicinal plants from South America. *Botanical medicine in clinical practice* (pp. 152-164), CABI International, Wallingford, UK.

Dey, U., Harlapur, S. I., Dhutraj, D. N., Suryawanshi, A. P.,Jagtap, G. P., Apet, K. T., & Wagh, S. S. (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(39), 4960-4971.

Dickinson, M. (2004).Resistance mechanisms in plants. In: *Molecular plant pathology* (pp. 160-174), Garland Science.

Edreva, A. (2004). A novel strategy for plant protection: Induced resistance. *Journal of Cell and Molecular Biology*, 3(2), 61-69.

Elsharkawy, M. M., & El-Sawy, M. M. (2015). Control of Bean common mosaic virus by plant extracts in bean plants. *International Journal of Pest Management*, 61(1), 54-59.

Flott, B. E., Moerschbacher, B. M., & REISENER, H. J. (1989). Peroxidase isoenzyme patterns of resistant and susceptible wheat leaves following stem rust infection. *New Phytologist*, 111(3), 413-421.

Fokkema, N. J. (1993). Opportunities and problems of control of foliar pathogens with micro-organisms. *Pesticide Science*, 37(4), 411-416.

Fu, Z. Q., & Dong, X. (2013). Systemic acquired resistance: turning local infection into global defense. *Annual Review of Plant Biology*, 64, 839-863.

Furumo, N. C., & Furutani, S. (2008). A simple method for assaying total protein, polyphenol oxidase and peroxidase activity from 'Kaimana' Litchi chinensis Sonn. *J. Haw. Pac. Agric*, 15, 1-7.

Gilchrist-Saavedra, L. (1997).Basidiomycetes. In: Gilchrist-Saavedra, L., Fuentes davila, G., Martinez-Cano, C., Lopez-Atilano, R.M., Duvieller, E, Singh, R.P., Henry, M., and, Garcia, A. (ED), *Practical guide to the identification of selected diseases of wheat and barley* (pp. 18-24). CIMMYT.

Goodman, R. N., Király, Z., & Wood, K. R. (1986). *The biochemistry and physiology of plant disease* (pp.433). University of Missouri Press. Columbia

Hammerschmidt, R., & Yang-Cashman, P. (1995). Induced resistance in cucurbits. In: *Induced Resistance to disease in plants* (pp. 63-85). Springer, Dordrecht.

Harris, C. A., Renfrew, M. J., & Woolridge, M. W. (2001). Assessing the risks of pesticide residues to consumers: recent and future developments. *Food Additives and Contaminants*, 18(12), 1124-1129.

Hatcher, P. E., & Paul, N. D. (2000). Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. *New Phytologist*, 146(2), 325-333.

Herman, M., & Williams, M. (2012). Fighting for their lives: plants and pathogens. *The Plant Cell*, 1, 1-17.

Hijwegen, T., & Verhaar, M. A. (1995). Effects of cucumber genotype on the induction of resistance to powdery mildew, *Sphaero thecafuliginea*, by 2, 6-dichloroisonicotinic acid. *Plant Pathology*, 44(4), 756-762.

Inbar, M., Doostdar, H., Sonoda, R. M., Leibee, G. L., & Mayer, R. T. (1998). Elicitors of plant defensive systems reduce insect densities and disease incidence. *Journal of Chemical Ecology*, 24(1), 135-149.

Janssen, A. M., Scheffer, J. J. C., & Svendsen, A. B. (1987). Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods. *Planta Medica*, 53(05), 395-398.

JOE, M. M., & Muthukumaran, N. (2008). Role of Certain Elicitors on the Chemical Induction of Resistance in Tomato against the Leaf Caterpillar Spodoptera litura Fab. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 36(2), 71-75.

Joseph, B., Dar, M. A., & 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(2), 56-59.

Kalidindi, N., Thimmaiah, N. V., Jagadeesh, N. V., Nandeep, R., Swetha, S., & Kalidindi, B. (2015). Antifungal and antioxidant activities of organic and aqueous extracts of Annona squamosa Linn. leaves. *Journal of Food and Drug Analysis*, 23(4), 795-802.

Karban, R., & Kuc, J. (1999). Induced resistance against pathogens and herbivores: an overview. *Induced Plant Defenses Against Pathogens and Herbivores*, 1-15.

Kassa, M. T., Menzies, J. G., & Mc Cartney, C. A. (2014). Mapping of the loose smut resistance gene Ut6 in wheat (*Triticum aestivum* L.). *Molecular breeding*, 33(3), 569-576.

Kazemi, N., Khavari-Nejad, R. A., 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(3), 402-407.

Kerby, K., & Somerville, S. (1989). Enhancement of spesific intercellular peroxidases following inoculation of barley with *Erysiphegraminis* f. sp. hordei. *Physiological and Molecular Plant Pathology*, 35(4), 323-337.

Kerby, K., & Somerville, S. C. (1992). Purification of an infection-related, extracellular peroxidase from barley. *Plant Physiology*, 100(1), 397-402.

Kessler, A., & Baldwin, I. T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, 53(1), 299-328.

Knoester, M., Pieterse, C. M., Bol, J. F., & Van Loon, L. C. (1999). Systemic resistance in Arabidopsis induced by rhizobacteria requires ethylene-dependent signaling at the site of application. *Molecular Plant-Microbe Interactions*, 12(8), 720-727.

Koch, E., Zink, P., Büssers, J., & Gossmann, M. (2013). 2-6 Association of the loose smuts of wheat and barley with fusaria and Acremonium-like fungi. *Endophytes for Plant Protection: The State of The Art*, 53.

Kolattukudy, P. E., Mohan, R., Bajar, M. A., & Sherf, B. A. (1992). Plant peroxidase gene expression and function. *Biochemical Society Transactions*. 20(2), 333-337.

Kumar, V., & Tyagi, D. (2013). Antifungal activity evaluation of different extracts of Bergeniastracheyi. *Int. J. Curr. Microbiol. App. Sci*, 2(7), 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(1), 85-89.

Lyon, G. (2007). Agents that can elicit induced resistance. In: Walters, D., Newton, A., &, Lyon, G. (ED). *Induced resistance for plant disease control: a sustainable approach to crop protection* (pp. 9-29). Blackwell Publishing, Oxford.

Mabona, U., Viljoen, A., Shikanga, E., Marston, A., & Van Vuuren, S. (2013). Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. *Journal of Ethnopharmacology*, 148(1), 45-55.

Madhusudhan, K. N., Nalini, M. S., Prakash, H. S., & Shetty, H. S. (2005). Effect of inducers against tobamo virus infection in tomato and bell pepper. *International Journal of Botany*, 1(1), 59-61.

Madhusudhan, K. N., Vinayarani, G., Deepak, S. A., Niranjana, S. R., Prakash, H. S., Singh, G. P., & Prasad, B. C. (2011). Antiviral activity of plant extracts and other inducers against tobamoviruses infection in bell pepper and tomato plants. *International journal of plant pathology*, 2(1). 35-42.

Mariita, R. M., Orodho, J. A., Okemo, P. O., Kirimuhuzya, C., Otieno, J. N., & Magadula, J. J. (2011). Methanolic extracts of Aloe secundiflora Engl. inhibits in vitro growth of tuberculosis and diarrhea-causing bacteria. *Pharmacognosy Research*, 3(2), 95-99.

Martinelli, J. A., Brown, J. K. M., & Wolfe, M. S. (1993). Effects of barley genotype on induced resistance to powdery mildew. *Plant Pathology*, 42(2), 195-202.

Mazid, M., Khan, T. A., & Mohammad, F. (2012). Medicinal plants of rural India: a review of use by Indian folks. *Global Journal of Pharmaceutical Sciences*, 2(3), 286-304.

Meyers, J. A., Sanchez, D. A. V. I. D., Elwell, L. P., & Falkow, S. T. A. N. L. E. Y. (1976). Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. *Journal of Bacteriology*, 127(3), 1529-1537.

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(4), 491-498.

Mohana, D. C., & Raveesha, K. A. (2007). Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *Journal of Agricultural Technology*, 4(1), 119-137.

Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., & Samiyappan, R. (2001). Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biology and Biochemistry*, 33(4-5), 603-612.

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 6(12), 1451-1474.

Newton, A. C., Flavell, A. J., George, T. S., Leat, P., Mullholland, B., Ramsay, L., & Thomas, W. T. (2011). Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. *Food Security*, 3(2), 141-178.

Pieterse, C. M., Van Wees, S. C., Van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., & Van Loon, L. C. (1998). A novel signaling pathway controlling induced systemic resistance in Arabidopsis. *The Plant Cell*, 10(9), 1571-1580.

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C., & Bakker, P. A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, 52, 347-375.

Pinelo, M., Manzocco, L., Nunez, M. J., & Nicoli, M. C. (2004). Solvent effect on quercetin antioxidant capacity. *Food Chemistry*, 88(2), 201-207.

Prasad, H. P., Shankar, U. A., Kumar, B. H., Shetty, S. H., & Prakash, H. S. (2007). Management of Bean common mosaic virus strain black eye cowpea mosaic (BCMV-BlCM) in cowpea using plant extracts. *Archives of Phytopathology and Plant Protection*, 40(2), 139-147.

Putievsky, E., Ravid, U., & Dudai, N. (1986). The influence of season and harvest frequency on essential oil and herbal yields from a pure clone of sage (Salvia officinalis) grown under cultivated conditions. *Journal of Natural Products*, 49(2), 326-329.

Quaglia, M., Ederli, L., Pasqualini, S., & Zazzerini, A. (2011). Biological control agents and chemical inducers of resistance for postharvest control of Penicillium expansum Link on apple fruit. *Postharvest Biology and Technology*, 59(3), 307-315.

Quijano, C. D., 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.

Palestinian Central Bureau of Statistics (PCBS). (2012). *Agricultural Statistics Survey annual report 2010-2011*. Retrieved from http://www.pcbs.gov.ps/Downloads/book1903.pdf.

Palestinian Central Bureau of Statistics (PCBS). (2007). *Agricultural Statistics annual report 2006-2007*. Retrieved from http://www.pcbs.gov.ps/Downloads/book1403.pdf

Rahber-Bhatti, M. H. (1988). Antifungal properties of plant leaf decoctions against leaf rust of wheat. *Pak. J. Bot*, 20(2), 259-263.

Rai, B. (2015). *Studies on leaf spot disease of ginger and its chemical management* (Doctoral dissertation).Uttar Banga Krishi Viswavidyalaya University, India.

Raj, D. S., Vennila, J. J., Aiyavu, C., & Panneerselvam, K. (2009). The hepatoprotective effect of alcoholic extract of Annona squamosa leaves on experimentally induced liver injury in Swiss albino mice. *International Journal of Integrative Biology*, 5(3), 182-186.

Rathod, M., Das, N. A. M. R. A. T. A., & Dhale, D. (2015). Antifungal activity of two medicinal plants against fungus Candida albicans. *Int J Pharma Bio Science*, 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(1), 36-39.

Reineke, A., Karlovsky, P., & Zebitz, C. P. W. (1998). Preparation and purification of DNA from insects for AFLP analysis. *Insect Molecular Biology*, 7(1), 95-99.

Resende, M. D., Nojosa, G. B. A., Cavalcanti, L. S., Aguilar, M. A. G., Silva, L. H. C. P., Perez, J. O., & Castro, R. M. (2002). Induction of resistance in cocoa against Crinipellis perniciosa and Verticillium dahliae by acibenzolar-S-methyl (ASM). *Plant Pathology*, 51(5), 621-628.

Reuveni, M., Agapov, V., & Reuveni, R. (1995). Induced systemic protection to powdery mildew in cucumber by phosphate and potassium fertilizers: effects of inoculum concentration and post-inoculation treatment. *Canadian Journal of Plant Pathology*, 17(3), 247-251.

Rogers, S. O., & Bendich, A. J. (1994). Extraction of total cellular DNA from plants, algae and fungi. In: Stanton B. Gelvin & Robbert A. Schilperoort(ED*), Plant molecular biology manual* (pp. 183-190). Springer, Dordrecht.

Runyoro, D. K., Matee, M. I., Ngassapa, O. D., Joseph, C. C., & Mbwambo, Z. H. (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complementary and Alternative Medicine*, 6(1), 11.

Sales, M. D. C., Costa, H. B., Fernandes, P. M. B., Ventura, J. A., & Meira, D. D. (2016). Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. *Asian Pacific Journal of Tropical Biomedicine*, 6(1), 26-31.

Schilling, L., Matei, A., Redkar, A., Walbot, V., & Doehlemann, G. (2014). Virulence of the maize smut *Ustilago* maydis is shaped by organ-spesific effectors. *Molecular Plant Pathology*, 15(8), 780-789.

Scorzoni, L., Benaducci, T., Almeida, A. M. F., Silva, D. H. S., Bolzani, V. D. S., & Gianinni, M. J. S. M. (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(3), 391-397.

Scott, I. M., Samara, R., Renaud, J. B., & Sumarah, M. W. (2017). Plant growth regulator-mediated anti-herbivore responses of cabbage (Brassica oleracea) against cabbage looper Tricho plusiani Hübner (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology*, 141, 9-17.

Seo, P. J., Lee, A. K., Xiang, F., & Park, C. M. (2008). Molecular and functional profiling of Arabidopsis pathogenesis-related genes: insights into their roles in salt response of seed germination. *Plant and Cell Physiology*, 49(3), 334-344.

Shabana, Y. M., Abdalla, M. E., Shahin, A. A., El-Sawy, M. M., Draz, I. S., & Youssif, A. W. (2017). Efficacy of plant extracts in controlling wheat leaf rust disease caused by Pucciniatriticina. *Egyptian Journal of Basic and Applied Sciences*, 4(1), 67-73.

Sharma, K., Bruns, C., Butz, A. F., & Finckh, M. R. (2012). Effects of fertilizers and plant strengtheners on the susceptibility of tomatoes to single and mixed isolates of Phytophthorainfestans. *European Journal of Plant Pathology*, 133(3), 739-751.

Sharma, K., Butz, A. F., & Finckh, M. R. (2010). Effects of host and pathogen genotypes on inducibility of resistance in tomato (Solanum lycopersicum) to Phytophthora infestans. *Plant Pathology*, 59(6), 1062-1071.

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security*, 4(3), 178-202.

Siddiqui, S., Bhardwaj, S., Khan, S. S., & Meghvanshi, M. K. (2009). Allelopathic effect of different concentration of water extract of Prosopsis juliflora leaf on seed germination and radicle length of wheat (Triticum aestivum Var-Lok-1). *American-Eurasian Journal of Scientific Research*, 4(2), 81-84.

Sowjanya, N. C., & Chary, C. M. (2012). Effect of plant extracts on the growth of Microsporum gypseum. *Journal of Phytology*, 40, 41-44.

Stout, M. J., Fidantsef, A. L., Duffey, S. S., & Bostock, R. M. (1999). Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, Lycopersicon esculentum. *Physiological and Molecular Plant Pathology*, 54(3-4), 115-130.

Svalheim, O., & Robertsen, B. (1990). Induction of peroxidases in cucumber hypocotyls by wounding and fungal infection. *PhysiologiaPlantarum*, 78(2), 261-267.

Thaler, J. S., Humphrey, P. T., & Whiteman, N. K. (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in plant science*, 17(5), 260-270.

Thaler, J. S., Stout, M. J., Karban, R., & Duffey, S. S. (1996). Exogenous jasmonates simulate insect wounding in tomato plants (Lycopersiconesculentum) in the laboratory and field. *Journal of Chemical Ecology*, 22(10), 1767-1781.

Tolman, J. H., McLeod, D. G. R., & Harris, C. R. (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(3), 915-921.

Ton, J., Van Pelt, J. A., Van Loon, L. C., & Pieterse, C. M. (2002). Differential effectiveness of salicylate-dependent and jasmonate/ ethylene-dependent induced resistance in Arabidopsis. *Molecular Plant-Microbe Interactions*, 15(1), 27-34.

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(4), 341-354.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., & Ryals, J. (1992). Acquired resistance in Arabidopsis. *The Plant Cell*, 4(6), 645-656.

Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science*, 44(6), 1920-1934.

van der Wolf, J. M., Michta, A., van der Zouwen, P. S., de Boer, W. J., Davelaar, E., & Stevens, L. H. (2012). Seed and leaf treatments with natural compounds to induce resistance against Peronospora parasitica in Brassica oleracea. *Crop Protection*, 35, 78-84.

Van Loon, L. C. (1997). Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology*, 103(9), 753-765.

Van Loon, L. C., Bakker, P. A. H. M., & Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*, 36(1), 453-483.

Varma, J., & Dubey, N. K. (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. *CurrentSscience*, 76(2), 172-179.

Vincelli, P. (2016). Genetic engineering and sustainable crop disease management: Opportunities for case-by-case decision-making. *Sustainability*, 8(5), 495-501.

Walling, L. L. (2000). The myriad plant responses to herbivores. *Journal of Plant Growth Regulation*, 19(2), 195-216.

Walters, D., & Heil, M. (2007). Costs and trade-offs associated with induced resistance. *Physiological and Molecular Plant Pathology*, 71(1-3), 3-17.

Walters, D. R., Havis, N. D., Paterson, L., Taylor, J., & Walsh, D. J. (2011). Cultivar effects on the expression of induced resistance in spring barley. *Plant Disease*, 95(5), 595-600.

Walters, D. R., Ratsep, J., & Havis, N. D. (2013). Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*, 64(5), 1263-1280.

Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., & Ryals, J. A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. *The Plant Cell*, 3(10), 1085-1094.

Webster, D., Taschereau, P., Belland, R. J., Sand, C., & Rennie, R. P. (2008). Antifungal activity of medicinal plant extracts; preliminary screening studies. *Journal of Ethnopharmacology*, 115(1), 140-146.

Wilcoxson, R. D. (1996).Loose Smut. In: Nielsen, J., &, Thomas, P. (ED), *Bunt and Smut diseases of wheat: concepts and methods of disease management* (pp. 33-47). CIMMYT.

Wunderle, J., Leclerque, A., Schaffrath, U., Slusarenko, A., & Koch, E. (2012). Assessment of the loose smut fungi (*Ustilago nuda* and *U. tritici*) in tissues of barley and wheat by fluorescence microscopy and real-time PCR. *European Journal of Plant Pathology*, 133(4), 865-875.

Zeller, W. (2006). Status on induced resistance against plant bacterial diseases. *Fitosanidad*, 10(2), 99-103.

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(1), 126-132.