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FOURIER-TRANSFORM INFRARED SPECTROSCOPY ANALYSIS AND ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACTS OF *MEDICAGO PARVIFLORA*, *SOLANUM NIGRUM*, *MELILOTUS ALBA* AND *MELILOTUS INDICUS* ON SOIL-BORNE PHYTOPATHOGENIC FUNGI

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Abstract

This research paper is based on the assessment to find the natural substitute of synthetic chemical fungicides and identify the functional group present in methanolic extracts of selected weeds. In this study fungicidal assessment is made of four noxious crop-weeds *Melilotus indicus, Melilotus alba, Medicago parviflora* and *Solanum nigrum*. The methanolic extract of all the selected weeds were screened for *In vitro* antifungal activities against the selected soil-borne fungal phytopathogens *Rhizoctonia solani, Rhizoctonia oryzae, Fusarium fujikuroi, Fusarium oxysporum, Pythium ultimum* and *Pyricularia oryzae.* Microspectrophotometric assessment technique is used for antifungal evaluation. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts were determined. Fourier-transform infrared spectroscopy (FTIR) method was used on methanolic extracts for their functional groups detection. Results indicated that growth of all above-mentioned fungal strains was significantly inhibited. The determined values of the weed extract ranged between 0.781-25 mg/mL while MFC values ranges were from 3.125 to 25 mg/ml. The extracts of *M. parviflora* have shown highest inhibitory activity (119.5%) against *P. ultimum* while *M. indicus* extract gave lowest suppression (97%) against *F. oxysporum*. FTIR spectroscopy of all selected weeds extracts confirms the presence of alkanes, alkynes, carboxylic acids, aldehydes and nitriles functional groups. These results support the potential use of these weed extracts in the management of crops fungal diseases.

Key words: Antifungal activity, Weed, Allelopathy, Methanolic extracts, FTIR.

Introduction

The world population has increased immensely i.e., almost double to 1960. It has been projected that the population will be 9.2 billion in 2050. Huge increase in population coupled with changing dietary habits of developing countries on the way to get high-quality food has led to food insecurity. The phenomenons will soon results into the increase in demand for food production up to 70% (Popp *et al.*, 2013). On the other hand, land is scarce and is available in very limited extent for additional agricultural purposes. To fulfill the increasing demand of food, the extension in agricultural land can only be made at cost of natural ecosystem and forests. So, there is the need to produce food on less land, with use of less energy, water, pesticide and fertilizer as compared to quantity of these being used today (Popp *et al.*, 2013). In agriculture practice, adopting cash crop plantation is a new practice and it has been expanding worldwide (Su *et al.*, 2016; Vongvisouk *et al.*, 2016). These cash cropping system enhances farmer's income considerably (Zhang *et al.*, 2017). Cash and potential crops yield enhancement is often linked to pest attack which leads to losses and increase in loss rates. About 50% loss in wheat, 80% in cotton are due to pests attack worldwide. The loss for wheat and cotton is estimated about 31%, 37%, for maize is 40% and for soybean ranges 26-29% (Popp *et al.*, 2013; Savary *et al.*, 2017).

The prevalence of plants diseases in ecosystem has increased intensely for the last two decades. The production quantity of many important trees and crops is decreased upto substantial level because of the plant pathogens (Fisher *et al.*, 2012). Though other contributing factors like planting of exotic species, wide area for monoculture are there but continuously increasing intensity and extent of the diseases is single biggest cause which have disturbed the natural ecosystem (Jactel *et al.*, 2009). It has been estimated that upto 90% agriculture yield loss are due to the attack of pathogenic fungi which is a major cause of plants diseases (Maninegalai *et al.*, 2011). The fungi persist for several years in soils by fabricating sclerotial and other forms of spores. Many precious host plants get damaged at large scale due to pathogenic diseases which includes stem rot, collar rot, root rot and leaf blights (Khan *et al.*, 2017). In Pakistan root rot and wilt disease infect almost all plants by several soil-borne fungi including *Fusarium* spp. Other fungi like *M. phaseolina* and *R. solani* make reduction in plants growth (Khan *et al.*, 2017; Usman *et al.*, 2014).

Use of pesticides is increasing day by day worldwide due to its effectiveness to control harmful organisms. The pesticides include a wide range of complexes including fungicide, rodenticide, insecticide, molluscicide, herbicide and nematicide etc. (Aktar *et al.*, 2009). The use of fungicide, herbicide, insecticide and other biotechnology products help to protect the crops from harmful insects, control numerous weed species and several plant diseases that affect the crops. The world food production would be waning, many vegetables and fruits would be in small stock and price of agriculture products would increase without the use of these vital skills of crop protection. Conversely, the extensive use of these pesticides unfortunately leads to serious environmental and health problems (Vikkey *et al.*,

2017). Increased rate of brain cancer (astrocytomas) and leukemia have been shown in children. Pregnant women have high miscarriage rate who are exposed to these pesticides (Hertz-Picciotto *et al.*, 2005). Pesticides cause inherited heart malformations and may also damage nervous system and lungs (Rallis *et al.*, 2014). In environment, pesticide can easily contaminate the water, air and ground, plant and animal life may be at risk when these pesticides run off from fields (El-Abbassi *et al.*, 2017).

Hence, worldwide researchers give more attention to find out some natural alternative and biological control to reduce or minimize the dependency on synthetic herbicides. Allelopathy can be considered as an effective natural alternative to synthetic pesticides. It can be explained as; the certain chemical compound produced by plants and microorganisms i.e. bacteria, fungi and viruses; these biomolecules released to the environment that influence the agricultural and ecological systems by stimulation or inhibition the growth of neighbor plants and microorganisms (Farooq *et al.*, 2011). These natural substances released by plants, called allelochemicals, have great potential to control plant pathogens compared to synthetic chemical compounds (Khan *et al.*, 2016). These natural allelochemicals have low environmental risk contrary to the synthetic chemical compounds and lower risk of persistence in environment. This is the reason to develop natural chemicals which are the alternative of conventional pesticides (El-Abbassi *et al.*, 2017). It will be a valuable advantage if these medicinal, antibacterial and fungicidal properties reside in noxious weeds. This research investigation is therefore, commenced to assess the worth of the common crops weed extracts against the soil-borne fungal phytopathogens.

Materials and Methods

Collection of plant material: The collection of four most toxic and noxious weeds species *Melilotus indicus* (L.) All., *M. alba* Desr., *Medicago parviflora* E. H. L. Krause and *S. nigrum* L. weeds leaf litter were made from different crops, fields, roadsides and meadows of Pakistan. Fresh leaves of weeds species were collected, separately packed in paper bags and then dried at 60°C in Biobase drying oven model BJPX-SUMMER for 24 hours approximately. Until the further experimental work the dried leaves were set aside in plastic bags and stored in air-tight box.

Extract preparation: All the samples were dried at 60°C for 24 hour in oven. A milling machine was used for grinding of dried leaves in powder form. The extracts of all samples were prepared accordingly as described by Basri & Fan (2005). About 100 g of powdered sample was extracted with 500 mL methanol for 48 hours in shaking incubator at 30°C. The extracts were filtered using Whatman No.1 filter and were concentrated under reduced pressure at 40°C using rotary evaporator. The crude extracts were allowed to dry at room temperature till constant weight. The extracts were redissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/mL for antifungal assay and were sterilized by filtering through 0.2 µm Millipore filter. The sterilized extracts were tested for sterility by taking 2 mL extract in 10 mL of sterile nutrient broth before incubation at 37°C for 24 hours. A sterile extract was indicated by the absence of turbidity in broth after incubation period. The extracts were stored at 4°C till further use.

Test Organisms and fungal inocula preparation: The fungal strains used in this study were obtained from Department of Microbiology, Quaid-e-Azam University, Islamabad and the fungal strains included *Pythium ultimum, R. solani, Pyricularia oryzae, Fusarium fujikuroi, R. oryzae* and *Fusarium oxysporum*. These strains were grown on sabaruad dextrose agar and were incubated at 30°C for 48 hours. Later on the suspension were prepared with an optical density (OD) of 0.1 at 630 nm.

Antifungal activity determination: A simple technique automated quantitative micro spectrophotometric assessment (Broekaert *et al.*, 1990) applied for *In vitro* antifungal activity measurement. Microtiter plates of 96 well at 630 nm used for growth inhibition measurement. A routine analysis of extracts under assay was performed with spore suspension (10 µL), extract (20 µL) and potato dextrose broth (PDB) (70 µL). The use of sterile distilled water (20 µL) having micro-cultures played a role of negative control. The Nystatin was applied as a positive control at 0.2 mg ml^{-1} (Satish *et al.*, 2007). An ELISA plate reader was used to measure absorbance at 630 nm of plates containing spore and sediments which were prepared at 27°C in 30 minutes. The absorbance was measured to record the growth after 2 days of incubation at 27°C through a triplicate assay for antifungal activity. The growth inhibition was determined by the following equation Broekaert *et al.*, (1990).

$$\text{Growth inhibition} = [(\Delta C - \Delta T) \div \Delta C] \times 100$$

where ΔC= Corrected absorbance of the control micro culture at 630 nm

ΔT= Corrected absorbance of the test micro-culture.

It has been noted that absorbance (at 630 nm) of micro-culture after 2 days minus measured absorbance after 30 minutes become equal to corrected absorbance of culture.

Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC): The minimal inhibitory concentration (MIC) values of plants extracts is known to be a lowest concentration of plants extracts resulting in a more than 90% growth inhibition as compared to the control during 48 hours. The MIC values of plants extracts were determined through a micro plate method (Eloff, 1998) after slight modification (dilution of solutions). This technique included the serial dilution of plant extract from crude extract in the range of 1/2 to 1/100 dilutions. The mixture contained 100 µL fungal spore suspension (2×10^6 spores mL^{-1} in fresh PDB) and each extract dilution (100 µL) in every well. The incubation of micro plates was carried out at 27°C for 48-72 hour in a triplicate experiment followed by spectrophotometric analysis (at 630 nm) with a micro plate reader. The comparison between growth in control wells and extract blank in un inoculated plates reveals the MIC values. Espinel-Ingroff *et al.*, (2002)

described *In vitro* fungicidal activity through incubation (72 hours) at 27°C, sub culturing (20 µL) from each positive well with no visible growth having more than 98% inhibition growth and the growth control onto PDA plates. The lowest extract concentration which did not result in fungal growth on used medium is considered as minimum fungicidal concentration.

Fourier transforms infrared spectrophotometer: For detection and confirmation of chemical bonds/functional groups in plants extracts the Fourier Transform Infrared (FTIR) Spectrophotometer were used which is perhaps the most powerful tools. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition. The absorption of light wavelength is key feature of chemical bond. To determine the chemical bonds in compound, the infrared absorption spectra can be interpreted. The KBr pellet technique was used for FTIR analysis (Karthishwaran *et al.*, 2010; Kumirska *et al.*, 2010). 10 mg of each weed leaf methanolic extract was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. A sophisticated, computer controlled FTIR spectrophotometer of Bruker, model Tensor27 with software version Opus65 equipped with ZnSe ATR were used to generate the FTIR spectra in the mid region of 400-4000 cm^{-1}.

Results and Discussion

In the present study, antifungal activity of plant crude extract (*M. indicus*, *M. alba*, *M. parviflora*, and *S. nigrum*) and their respective dilutions was conducted. Among these plants; the extract of *M. parviflora* showed highest activity against *Pythium ultimum* (Fig. 2) and the lowest activity was shown by *M. indicus* extract against *Fusarium oxysporum* (Fig. 1). Overall all of the extract showed potent activities against all fungal strains. Among the fungal strains, *P. ultimum*, *R. solani* and *F. oxysporum* were highly susceptible compared to the rest of the strains tested. *R. oryzae*, *F. fujikoroi* and *P. oryzae* were slightly resistant compared to the other strains tested. The positive control Nystatin also showed potent activity against all fungal strains as shown in Fig. 1-4. The highest activity was shown by Nystatin against *P. ultimum* and *P. oryzae*.

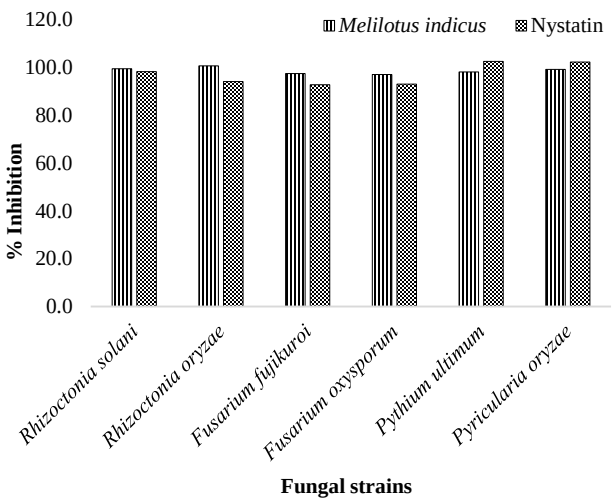

Fig. 1. Growth inhibition of fungal strains by crude extracts of *Melilotus indicus.*

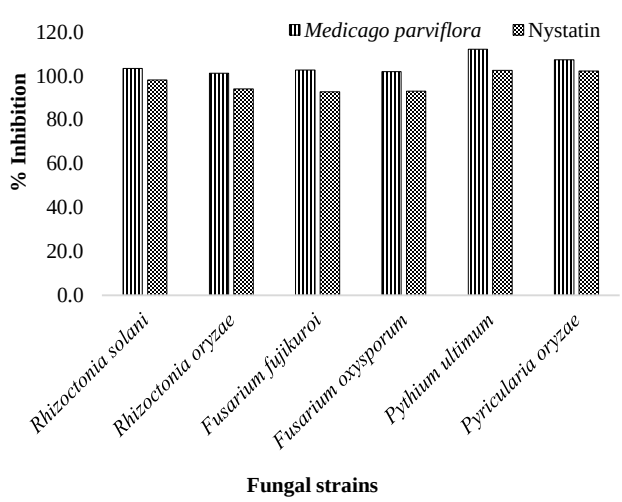

Fig. 2. Growth inhibition of fungal strains by crude extracts of *Medicago parviflora*.

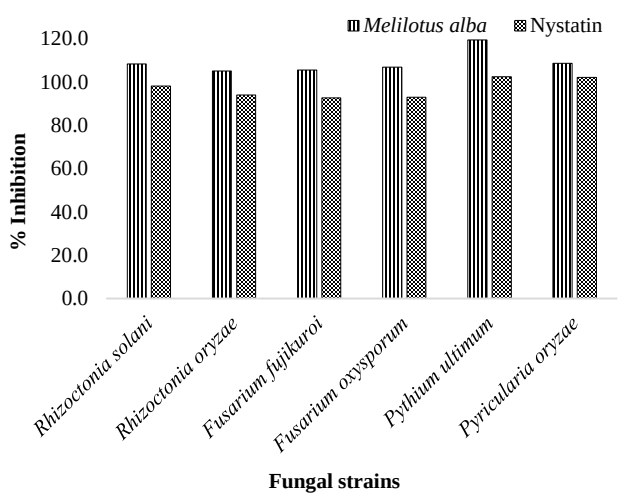

Fig. 3. Growth inhibition of fungal strains by crude extracts of *Melilotus alba*.

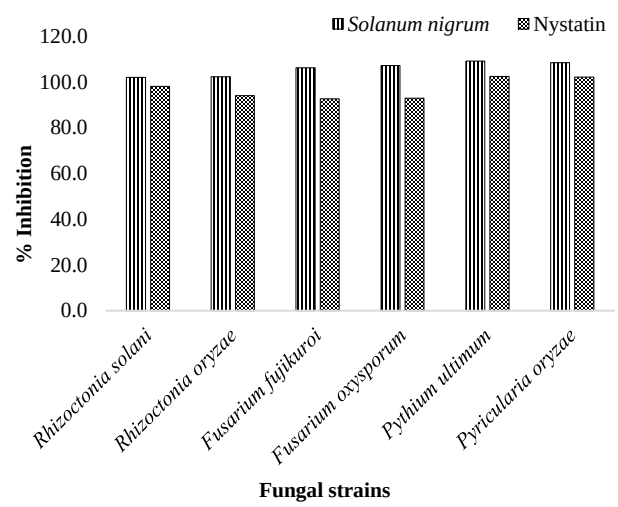

Fig. 4. Growth inhibition of fungal strains by crude extracts of *Solanum nigrum*.

Table 1. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of crude extracts of plants names.

Names	MIC (mg/mL)						MFC (mg/mL)					
	Pythium ultimum	***Rhizoctonia solani***	***Pyricularia oryzae***	***Fusarium fujikuroi***	***Rhizoctonia oryzae***	***Fusarium oxysporum***	***Pythium ultimum***	***Rhizoctonia solani***	***Pyricularia oryzae***	***Fusarium fujikuroi***	***Rhizoctonia oryzae***	***Fusarium oxysporum***
Melilotus indicus L.	12.5	12.5	0.78	0.78	3.125	12.5	25	6.25	3.125	3.125	3.125	25
Melilotus alba Desr.	12.5	12.5	0.78	0.78	6.25	12.5	25	25	3.125	3.125	12.5	25
Medicago parviflora E.H.L. Krause	6.25	6.25	0.78	0.78	1.56	12.5	-	12.5	3.125	3.125	3.125	25
Solanum nigrum L.	3.125	6.25	0.78	0.78	1.56	6.25	6.25	12.5	3.125	3.125	3.125	12.5
Nystatin* (U/mL)	1250	1250	156.3	156.3	625	1250	2500	2500	312.5	312.5	1250	2500

Nystatin*: Nystatin was used in units/ml

The minimum inhibitory concentration (MIC) of all extracts was determined against all tested fungal strains. About seven dilutions were prepared and were tested for MIC in triplicate. The dilution of extract ranges from (0.781-25 mg/mL) and the positive control Nystatin was (156.25-5000 units/mL). The minimum inhibitory concentration of all the extracts varied from strain to strain. *F. oxysporum*was the most susceptible strains among all strains tested for MIC. The MIC shown by all extracts against *F. oxysporum* was in the range of 6.25-12.5. On the contrary the *P. oryzae* and *F. fujikuroi* were the most resistant strains among all tested strains for MIC. Most of the extracts showed least minimum inhibitory concentration against these strains. The MIC of all extracts against these strains was 0.781 mg/mL. The positive control Nystatin also showed potent activities against fungal strains tested. The MIC of Nystatin was in range of 15.25-1250 U/mL as shown in Table 1.

The minimum fungicidal concentration of all the crude extracts was established. The MFC values were in the range of 3.125-25 mg/mL. The highest minimum fungicidal activity was observed against *P. oryzae* and *F. fujikuroi* that was 3.125 mg/mL. On the other side, no MFC was shown against *P. ultimum* species by extract *M. parviflora* and in addition all of the extracts showed poor MFC against *P. umultimum* and *F. oxysporum* with a concentration of 6.25-25 mg/mL as shown in Table 1.

Weeds of notorious nature and allelopathic potential with limited reported antifungal activity were selected for authentication of their use against fungal pathogens. For this purpose, the top most noxious and allelopathic weeds were selected. These notorious weeds included *M. indicus, M. alba, M. parviflora* and *S. nigrum*. Methanolic extracts of above mentioned plants leaves were tested against soil-borne phytopathogenic fungi, i.e., *R. solani, R. oryzae, F. fujikuroi, F. oxysporum, P. ultimum and P. oryzae*. Methanolic fractions exhibited more promising results than aqueous fractions in suppressing the fungal growth (Buch & Arya, 2017).

R. solani (Ceratobasidiaceae family), a form of rot, commonly found in most soils and cause several disease in almost all agricultural crops (Feng *et al.*, 2017). Susceptibility of all cultivars to this pathogen make them vulnerable for pest attacks. *R. solani* effectively was controlled with the application of systemic fungicides and antibiotics (e.g. jinggangmycin or validamycin) (Feng *et al.*, 2017). Biological control of *R. solani*are reported from about 100 years through microorganisms mostly by using fungal strains e.g. *Trichoderma* spp.,*Chlonosta chysrosea* and *Coniothyrium minitans* etc. (Daguerre *et al.*, 2017) bacterial strains like *Streptomyces* strains, *Bacillus sp. ,Pseudomonas* spp. etc. and bacterial virus strains isolated from the endosphere or rhizosphere (Ahsan *et al.*, 2017). Antifungal activities of different medicinal plant extracts diluted with 50% Acetone e.g.,*Trachystemon orientalis, Smilax excelsa,* siam weed and wild sunflower etc. from Turkey and Sir Lanka have been reported against *R. solani* (Onaran & Sağlam, 2016). This research study was focused on the top most noxious weeds of agricultural system which have already affect the yield production worldwide including Pakistan. *M. parviflora* is herbaceous weed distributed in harvested crops and fields, never before reported its medicinal, antimicrobial or any other ecological properties. *M. parviflora*showed highest antifungal activity against *R. solani* strain (Fig. 2) succeeded by *M. alba* (Fig. 3), *S. nigrum* (Fig. 4) and *M. indicus* respectively (Fig. 1).

Rhizoctonia oryzae commonly known as teleomorph or Waiteacircinate belong to family Ceratobasidiaceae. This pathogenic fungus causes several diseases in crops like sheath spot of rice, root rot and crown of wheat, stalk rot of maize and root rot of barley (Doussoulin *et al.*, 2016). Pesticides extracted from plants are favored for risk reduction associated with chemical control techniques. Clove, neem, rosemary and pelargonium extracts suppressed fungal growth including *R. oryzae* (San & Matsumoto, 2011). The reported plants extracts used for biological control of soil-borne pathogenic fungi *R. oryzae*have economical and medicinal application while the plants used for antifungal activities are toxic weeds. Results indicated that *M. parviflora* (Fig. 2) showed highest antifungal activity against *R. oryzae*.*S. nigrum* leaf extracts were found to give the second-best suppression against the tested fungi (Fig. 4).

Many *Fusarium* spp., are distributed worldwide and have economic importance by producing toxic and deadly secondary metabolites to environment which leads to cause diseases in plants, animals and as well in human (Leslie & Summerell, 2006). Known reported species included *F. poae*, *F. verticillioides* and members of the *F. solani* species complex (FSSC), *F. oxysporum* species complex (FOSC) and the *F. graminearum* species complex (Streit *et al.*, 2012). However, most agricultural plants are host to *F. fujikuroi*. *F. fujikuroi*is hemibiotrophic fungus and can be transmitted to host vertically (seed-borne) or horizontally (soil- or aerial-borne, or through wounds) where it causes show root, stalk and ear rot, as well as wilting, stalk thinning and reduced aerial and root growth (Wu *et al.*, 2011). The other sturdiest pathogenic and globally distributed fungus of this group is *F. oxysporum* (soil-borne ascomycete). Many commercially cultivated harvests and some other crops are host to *F. oxysporum* strains which infected and killed the harvested crops. *F. oxysporum* is transmitted to host through root, block its vascular system and stops transportation process which cause flaccid, streak and eventually the plant die. Additionally, *F. oxysporum* covers outside plant kingdom, into Animalia and deceitful human pathogen, reported in immune-compromised patients the well-identified agents producing invasive fungal diseases. These fungal infections in mammals are eventually fatal as resistance is developed in *F. oxysporum* against available antifungal drugs.

Previous research studies have already been reported many *In vitro* efficacy of different higher plant extracts and their constituents examined for successful biocontrol of *F. fujikuroi* and *F. oxysporum* because of their fungi toxicants nature with less harming ecosystem capability due to their biodegradability (Bashar & Chakma, 2014). Among all the tested plants extracts only few showed 100 % suppress the mentioned soil-borne fungi i.e. *F. fujikuroi and F. oxysporum*. The present research study is to assess the potential of methanolic extract of allelopathic weeds leaves extracts to control the pathogenic fungus *F. fujikuroi* and *F. oxysporum.* The result of obtained from research work indicated that *S. nigrum* and *M. Parviflora* showed 106.2% (Fig. 4) and 105.5% (Fig. 2) suppression respectively to the tested *Fusarium* species.

*Pythium ultimum*is also a soil-borne pathogenic fungus of family Pythiaceae. *P. ultimum* causes a wide range of problems like damping-off, seedling blight root rot and stem rot diseases of hundreds of diverse plant hosts including corn, soybean, carrot, cucumber, melon, potato, wheat, fir, and many ornamental species(Farr & Rossman, 2014). *P. ultimum* is abundantly found in soil moisture and high soil temperature regions of the world. It is very problematic to control soil-borne pathogenic *P. ultimum* only with fungicides like mefenoxam, thiadiazole, etridiazole, propamocarb, dimethomorph, and phosphonates and moreover this method is also uneconomical (Gholve *et al.*, 2014). Biologically *P. ultimum* controlled through microorganisms which includes some bacterial strains of *Bacillus*, *Streptomyces* and *Pseudomonas* species and fungal strains like *Trichoderma*, *Gliocladium* and *Candida* (Berendsen *et al.*, 2012). Garlic extracts and essential oils of T*hymus vulgaris*, *Lavandula* spp. And *Mentha piperita* and methanol extract, obtained from *Tagetes patula* plant, and controlled *P. ultimum* (Cruz *et al.*, 2013). The reported plants used for antifungal assessment are either ornamental or have agricultural importance but here in this research the plants selected for antifungal assessment are totally unwanted plants. *M. parviflora* showed highest (Fig. 2) while *M. indicus* showed lowest activity (Fig. 1) against *P. ultimum* among all tested weeds extract.

Pyricularia oryzae is a virulent species of family Magnaporthaceae employ a hemibiotrophic stratagem to enter host and sequentially establishing infection at biotrophic and necrotrophic stages (Marcel *et al.*, 2010). Rice blast is the most destructive reported problem worldwide caused by *P. oryzae*, which lead to a notable reduction in yields about 30% of rice production (Hosseyni-Moghaddam & Soltani, 2013). Previous research studies have established fungal resistance to chemical treatments and genetic manipulation have been established. Turmeric, garlic, van tulsa and Ginger extracts are reported as significant antifungal agent against *P. oryzae* (Khanzada & Shah, 2012). In this current investigations, *M. parviflora* and *S. nigrum* leaf extracts show highest suppressive activity respectively against *P. oryzae* (Figs. 2 & 4).

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 2. The FTIR spectrum profile of *M. indicus*, *M. parviflora*, *M. alba* and *S. nigrum* were illustrated in the Figs. 5, 6, 7 and 8 respectively. FTIR spectrum confirmed the presence of alkanes, alkynes, aldehydes, carboxylic acids, and Nitriles in methanolic extracts of all selected weeds. The peak at 2984.87 and 2802.98 cm-1 refers to the presence of alkanes (H-C-H Asymmetric & Symmetric Stretch). A peak of 2195.4-2103.36 cm-1 showed the presence of alkynes (C≡C Stretch). The peak at 2984.87and 2414.62 cm-1 corresponds the carboxylic acid group (H-bonded O-H Stretch). A peak at 2849.29-2802.98 and 2739.04-2701.5 cm-1 denotes the aldehydes (C-H Stretch off C=O). The peaks of 2290.3-2202.81 Nitriles (C≡N Stretch).

Table 2. FTIR absorption ranges and functional groups of methanolic extracts of selected weeds.

Functional group names	Absorption ranges of *Melilotus indicus*	Absorption ranges of *Medicago parviflora*	Absorption ranges of *Melilotus alba*	Absorption ranges of *Solanum nigrum*	Type of vibration
Alkanes	2973.52 - 2848.98	2972.22-2841.63	2976.77- 2845.17	2984.87- 2802.98	H-C-H Asymmetric & Symmetric Stretch
Alkynes	2140.32 -2103.36	2180.87-2132.78	2195.4- 2113.69	2184.08- 2119.87	C≡C Stretch
Carboxylic acids	2973.52-2491.38	2972.22-2423.47	2976.77-2439.74	2984.87- 2414.62	H-bonded O-H Stretch
Aldehydes	2848.98 & 2731.3-2710.14	2841.63 & 2730.91-2705.66	2845.17 & 2739.04- 2701.5	2849.29-2802.98 & 2733.72-2713.38	C-H Stretch off C=O
Nitriles	2266.89-2207.1	2289.46- 2233.69	2290.3-2237.03	2257.15-2202.81	C≡N Stretch

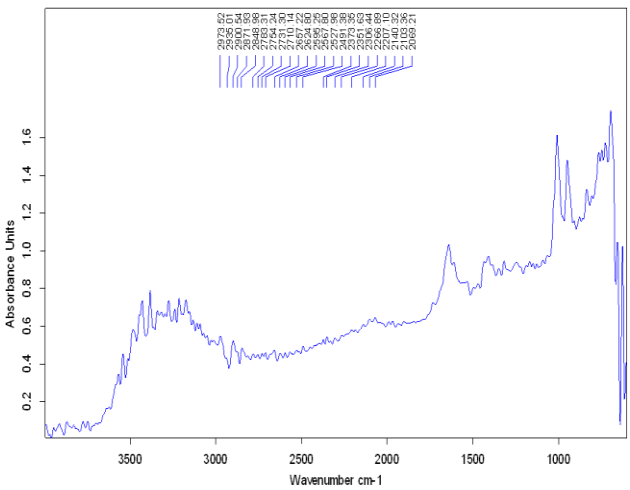

Fig. 5. Fourier transform Infrared spectrum analysis of *Melilotus indicus*.

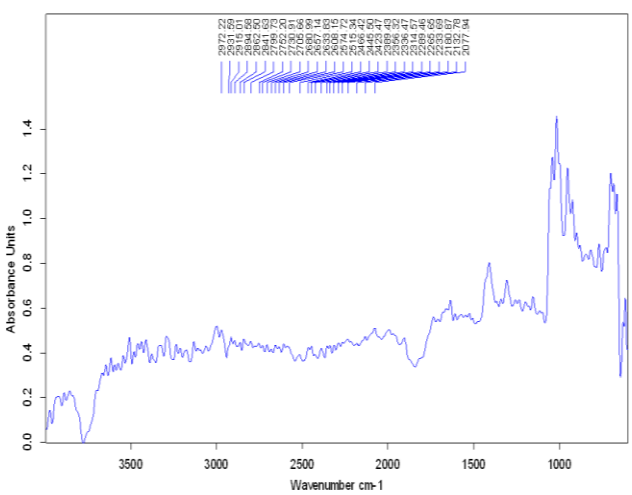

Fig. 6. Fourier transform Infrared spectrum analysis of *Medicago parviflora*.

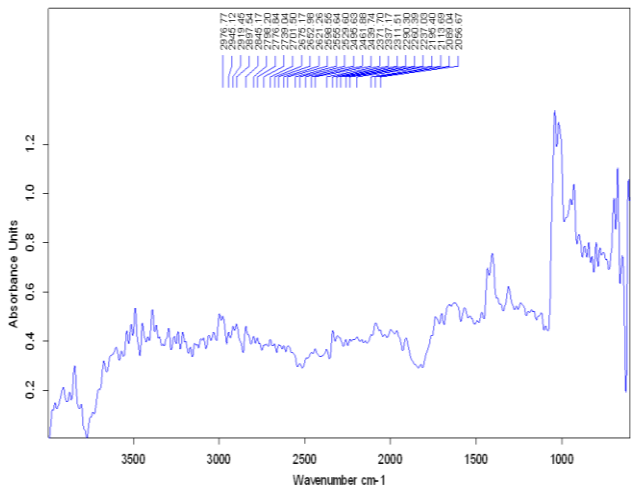

Fig. 7. Fourier transform Infrared spectrum analysis of *Melilotus alba*.

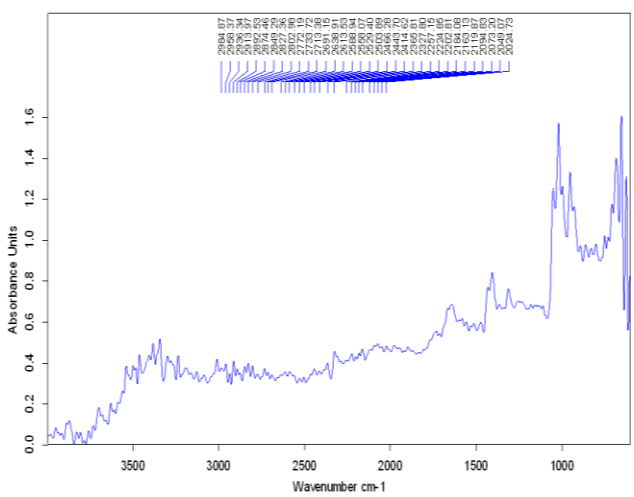

Fig. 8. Fourier transform Infrared spectrum analysis of *Solanum nigrum*.

Carboxylic acids have a carbonyl group and an alcohol group they share some basic physico-chemical properties with aldehydes, ketones and alcohols (DeRuiter, 2005). Carboxylic acid esters were synthesized and evaluated *In vitro* against *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus niger*. All carboxylic acid ester derivatives exhibited higher antifungal activity than fluconazole (Nam *et al.*, 2004). Aldehyde is an organic compound containing a formyl group. Amongst the aldehydes, glutaraldehyde is an important dialdehyde used as sterilant and disinfectant. Glutaraldehyde has a broad spectrum of activity against bacteria, fungi, and viruses. Formaldehyde and Cinnamaldehyde are also used as fungicide (Lamba, 2007). Ten bis (alkylpyridinium) alkane compounds were tested for antifungal activity against yeasts and molds. This study has identified *In vitro* antifungal activities of novel bisalkypyridinium alkane compounds. The compounds were more potent against different strains of fungi then molds (Chen *et al.*, 2010). A large number of different nitriles have high insecticidal and acaricidal activity. The halogenated aromatic dinitriles exhibit outstanding biological activity as fungicides, bactericides and nematocides (Melnikov *et al.*, 2012).

Fungitoxicity effects of the phyto-extracts indicate the potentials of selected plant species as a source of natural fungicidal substantial. The functional groups of extracts exhibit significant fungicidal properties that support the importance of these plants in agro-ecosystem. In the case of fungal infection, these mechanisms include synthesis of bioactive organic compounds (Rongai *et al.*, 2012) and antifungal proteins (Morrissey & Osbourn, 1999) and peptides (Boyd & Tucker, 1998; Dissanayake & Jayasinghe, 2013). Thus, the weeds can be applied to the plants for fungicidal purposes, for example, by spraying the plants with aqueous or organic solvent.

Conclusion

All the selected and assessed unwanted plants for fungicidal activities show strong fungal inhibition. Results showed that *Medicago parviflora* and *S. nigrum* have strong suppressive potential against fungal growth of all tested soil-borne phytopathogenic fungi. Identified functional groups of plant extracts by FTIR analysis, also support the antifungal potential of selected weeds. Outcome of this present research study could be an

important step towards the possibilities of using natural unwanted plant products as biopesticides in the control of plant diseases caused by *R. solani, R. oryzae, F. fujikuroi, F. oxysporum, P. ultimum*and *P. oryzae.* Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity.

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