Pak. J. Bot., 55(1): 403-409, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-1(28)

PHYTO-CHEMICAL ANALYSIS OF PLANT-PRODUCTS AND THEIR INFLUENCE AGAINST SOIL-BORNE PHYTOPATHOGENS

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Abstract

Plants are able to produce various chemical substances in order to protect their body from several abiotic and biotic stresses. These phyto-chemicals are secondary metabolites that can inhibit pathogenic growth by terminating their cellular body. In present studies, it is evaluated that plant products like aerial part of Alfalfa, Rice husk and wheat straw comprised phyto-chemicals with different strength. Study showed that alfalfa contains greater amount of phyto-chemicals such as total phenols, flavonoids and higher percentage of antioxidants. It was also able to inhibit the mycelial growth of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* by its methanolic and chloformic extracts. Beside alfalfa, methanolic extract of wheat straw exhibited 100 % eradication of *R. solani* and *F. oxysporum* as comprised second highest measures of phyto-chemicals. Rice husk contained lesser amount of total phenols, total flavonoids and lowest percentage of antioxidant. Rice husk also showed minimum inhibition percentage of phytopathogens as compared to the other plant extracts. It is concluded that all plant products contain different strengths of phytochemicals and have ability to inhibit the mycelial growth of phytopathogens to protect their plant body against infections. It is also concluded that among above mentioned plant species, extracts of alfalfa and wheat straw showed greater results of phyto-chemicals as well as inhibition percentage of pathogens.

Key words: Phyto-Chemicals, Antifungal activity, Soil-borne phytopathogens.

Introduction

Plant diseases are the source of direct destruction in agriculture field which caused by various pathogenic microorganisms including fungi, bacteria, nematodes etc. these pathogens are broadly distributed in every global ecosystem. Among various pathogenic microbes, soil-borne phytopathogenic fungi can survive even during unfavorable environmental conditions by producing resting bodies such as sclerotia which are greatly resistant to heat, chemicals and bio-degradation (Coley-Smith & Cooke, 1971).

Plant root diseases caused by soil-borne pathogenic fungi are more common in vegetable crops comprising beans, carrot, lettuce, onion etc. (Abawi *et al*., 1985; Sherf & MacNab, 1986; Hall, 1991). High incidence of root diseases leads to severe losses in agriculture field and economy. These losses are due to various soil-borne pathogens such as *Rhizoctonia solani*, *Pythium ultimum*, (Roberts *et al*., 2005) *Macrophomina phaseolina* (Ghaffar *et al*., 1964) and *Fusarium* spp. (Booth, 1971). Economically vital crops are damaged by these individual pathogens or their combined effect that results in reduced yield with poor quality (Agrios, 1988). For example, a group of wilt pathogens such as *Fusarium oxysporum*, *Verticillium dahliae* etc. are responsible for wilt disease in plant when enter in vascular tissues by endodermis and block the water passage by moving in xylem cells to aerial portions of the plant (Beckman, 1987). Disruption of water channel cause death of plant. Mycelium of such pathogens like *Rhizoctonia* or *Gaeumannomyces* can survive in soil and spread towards neighboring healthy plant roots from dead or infected plant parts (Bailey *et al*., 2005; Bailey & Gilligan, 1999, 2004). According to Davidson *et al*., (2005), *Phytophthora ramorum* is a pathogen of sudden death in oak plant and this pathogen can move into soil and water bodies of ecosystems. Although, mycelium of several soil-borne phytopathogens move and extend directly into soil profile. Such as, species of genus *Rhizoctonia* spread quicker in soil having greater porosity with superior pores (Otten *et al*., 1999; Harris *et al*., 2003). However, development of disease is depended on interrelationship among pathogen, susceptible host and favorable environment.

Approximately 300,000 plant species have been revealed to comprise several phytochemicals with various structures and properties (Lattanzio, 2013). These phytochemicals are divided into two main groups that includes primary metabolites like carbohydrates, lipids, proteins etc. and secondary metabolites such as alkaloids, terpenoids, and phenolic compounds. Primary metabolites serve growth and development of plants while secondary metabolites aid to develop defense mechanisms of plant against environmental stress, pollutants, pathogens, and other external pressures. (Bernards, 2010). Among all these different phytochemicals, phenolic contents and flavonoids are revealed as main classes of biochemicals that contain substantial medicinal property for living organisms.

The antioxidant activity of phenolic contents and flavonoids is based on the existence of the hydroxyl (-OH) group in the plant species. Moreover, its position is also very important to influence on the ability of free radical scavenging activity (Meenakshi *et al*., 2009; Balasundram *et al*., 2006). Consequently, many scientists have reported several medicinal activities of phenolic compounds in plant species for example antioxidants, antimicrobial, antidiabetic and anticancer (Ferguson, 2001; Cai *et al*., 2004; Christ-Ribeiro *et al*., 2019).

At present, several toxic constituents are entering in the food chain of plants and animals that leads to the various diseases in living organisms. The reason behind this is the attack of free radicals of reactive oxygen species on fatty acids, lipids, proteins, DNA, etc. that results in the damage of cell membranes by quick destructive chain reaction (Saravanan *et al*., 2020). Thus, these damages by free radicals can be obstructed by phenolic compounds and flavonoids (Abdel-Hameed, 2009; Dewanto *et al*., 2002). Therefore, inspection and description of phytochemicals in the plant species are very important to reveal their activities against various bio-threats. In the present studies, three different plant products are selected according to their properties such as aerial parts of Alfalfa, Rice husk, and Wheat straw for scrutinizing their phytochemicals against different soil-borne pathogens.

Materials and Methods

Different plant products were collected from different localities of Sindh. Like, aerial parts of Alfalfa (Karachi), Rice husk and Wheat straw (Thatta). Plants products were washed, air died and powdered by electric grinder for *In-vitro* studies.

Preparation of plants extracts: Extracts from three different plant products were made with aqua and various organic solvents like methanol, petroleum ether and chloroform. Twenty five g powder of each product was soaked in 100 mL of different solvents for 24 hours at room temperature (25 ± 2 ℃). Solvents were filtered with Watman no. 1 filter paper and evaporated to make final (standard) volume 25 mL by using water bath (Barreto *et al*., 2002).

Phyto-chemical analysis: Bio-chemical analysis including total phenolic contents, total flavonoids and antioxidant activity (by DPPH) of ethanolic plant extracts have been carried out by following methods.

1. Total phenolic contents: Total phenolic contents in plant products was determined by the method of Iqbal *et al*. (2005). Crude ethanolic plant extracts were diluted by the ratio of 1:9. One mL of each extract was mixed with five mL of folin reagent (diluted with distilled water as 1:9). Four mL of 7.5% Na_2CO_3 was added in solution mixture and stand for 30 min at the room temperature. The absorbance of solutions were recorded at 765nm against reagent blank by spectrophotometer (JENWAY 6305). The extent of total phenolic contents was calculated by the standard curve using mg of galic acid per ml.

2. Total flavonoid contents: The total flavonoid content was determined according to the aluminum chloride-colorimetric method (Lin *et al*., 2007) with some modifications. 100 µL of diluted ethanolic extract (1:9) was added in 0.3 mL of 5% $NaNO_2$ and stand for 5 min at room temperature. After the particular time, 0.3 mL of 10% $AlCl_3$ was mixed in the solution mixture and followed by the addition of 1 M NaOH solution. The absorbance of the solution mixture was measured at 415 nm against reagent blank by spectrophotometer (JENWAY 6305). The amount of total flavonoid contents was further calculated by the standard curve as mg of quercetin.

3. Antioxidant activity of plant extracts using DPPH: The antioxidant activity of the ethanolic plant extracts was determined using DPPH free radical scavenging activity by the slightly modified method of Abdille *et al*., (2004). 1 mL of diluted plant extract (1:9) was mixed with 2 mL of 0.002% methanolic solution of DPPH. The solution mixture was stand for 30 min at 25°C under dark condition. The control blank solution was made using methanol instead of sample extract. The absorbance of the sample solutions was recorded at 515 nm against reagent blank by spectrophotometer (JENWAY 6305). The inhibition percentage (%) of radical scavenging activity of plant extracts was calculated by the following formula:

$$\text{Inhibition (\%)} = \frac{\text{absorbance of control solution} - \text{absorbance of sample}}{\text{absorbance of control solution}} \times 100$$

Isolation of soil-borne phytopathogenic fungi: Four different plant pathogenic fungal species such as *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* were isolated from the field soil of Federal Urdu University of Arts, Science and Technology, Karachi. Isolation of pathogens were carried out by different methods such as isolation of *Fusarium* spp. were carried out by serial dilution technique (Waksman, 1922), baiting technique was used for *R. solani* (Papavizas & Davey, 1962), while, *M. phaseolina* was isolated via wet sieving method (Shaikh & Ghaffar, 1975). Isolated fungal species were grown on potato dextrose agar (PDA) to make pure cultures and preserved for further use.

Antifungal inspection of plant extracts: Plant extracts of aqua and different solvents were examined against isolated plant pathogenic fungal species by food poison technique (Nene & Thapliyal, 1979). Each extract was added in separate sterilized PDA medium with a ratio of 1:9. The agar disc of 6mm of each pathogen from pure culture was inoculated in already prepared PDA plate (9 inch) of each extract. Petri plates were incubated at 28 ± 2℃ for 7 days and checked the mycelial growth of pathogen. The inhibition percentage of pathogens were measured by using following formula:

$$\text{Inhibition Percentage of mycelial growth (\%)} = \frac{N1 - N2}{N1} \times 100$$

where; N1 was represented as the mycelial growth of pathogen in control plate and N2 was the growth of pathogen in treated PDA medium.

Statistical analysis of all investigations were carried out through analysis of variance (ANOVA) by SPSS 20.

Results

Present study showed significant amounts of total phenols (F = 161.257, p<0.000), total flavonoids (F = 3087.29, p<0.000) and antioxidant activity (F = 107.456, p<0.000) in different plant products as shown in Table 2. Among all plant products, alfalfa comprised greater amount of total phenolic contents (1.85 ± 0.04 mg/g), total flavonoids (3.61 ± 0.03 mg/g) as well as percentage of antioxidant activity (42.84 ± 1.67%). Whereas, wheat straw showed second highest measures of total phenolic contents (1.26 ± 0.04 mg/g), flavonoids (1.00 ± 0.05 mg/g) and percentage of antioxidant activity (30.73 ± 0.62%) after alfalfa (Fig. 1). Instead, rice husk exhibited lowest amounts of total phenolic contents (0.87 ± 0.03 mg/g), flavonoids (0.04 ± 0.01 mg/g) and antioxidant activity (19.96 ± 0.68%) as compare to the other plant product extracts.

In the present research, extracts from various plant products by different organic solvents have been studied to reveal their effects against these phytopathogens. Results showed that methanolic extracts of all plant products revealed highest inhibition percentage of pathogens which is followed by chloroform (Table 1). Wherein, 100 ± 0.00% inhibition of *R. solani* was exhibited by methanolic extracts of alfalfa and wheat straw as shown in Table 1. In case of *M. phaseolina* which can survive in very harsh conditions by producing scloretia have been showed highest inhibition percentage by methonolic extract of alfalfa (85.18 ± 4.50%) as compare to other organic solvent and aqueous extracts. Wheat straw extracts by methanol and rice husk extract by chloroform significantly inhibited mycelial growth of *F. oxysporum* (100 ± 0.00%). Whereas, *F. solani* was significantly eradicated by chloroformic extract of alfalfa (100 ± 0.00%).

Our results revealed that all plant product extracts possessed inhibitory effects against different plant pathogens as they comprised varied measures of phytochemicals. It is also exhibited that alfalfa presented greater inhibitory properties for pathogens by means of containing highest amount of total phenolic contents, total flavonoids as well as higher percentage of antioxidant activity among all plant products. Consequently, alfalfa exhibited highest significant inhibition of mycelial growth of *M. phasealina* (F = 12.34, p<0.002), *R. solani* (F = 15.73, p<0.001), *F. oxysporum* (F = 11. 52, p<0.003) and *F. solani* (F = 177.74, p<0.000). Whereas, rice husk showed good results after wheat straw. Although, rice husk also revealed considerable results of fungal inhibition (Table 3) such as *M. phasealina* (F = 4.09, p<0.049), *R. solani* (F = 75.72, p<0.000), *F. oxysporum* (F = 72.43, p<0.000) and *F. solani* (F = 9.86, p<0.005). While, wheat straw significantly terminated mycelial growth of pathogens after alfalfa *M. phasealina* (F = 6.08, p<0.014), *R. solani* (F = 15.90, p<0.001), *F. oxysporum* (F = 394.35, p<0.000) and *F. solani* (F = 19.00, p<0.001).

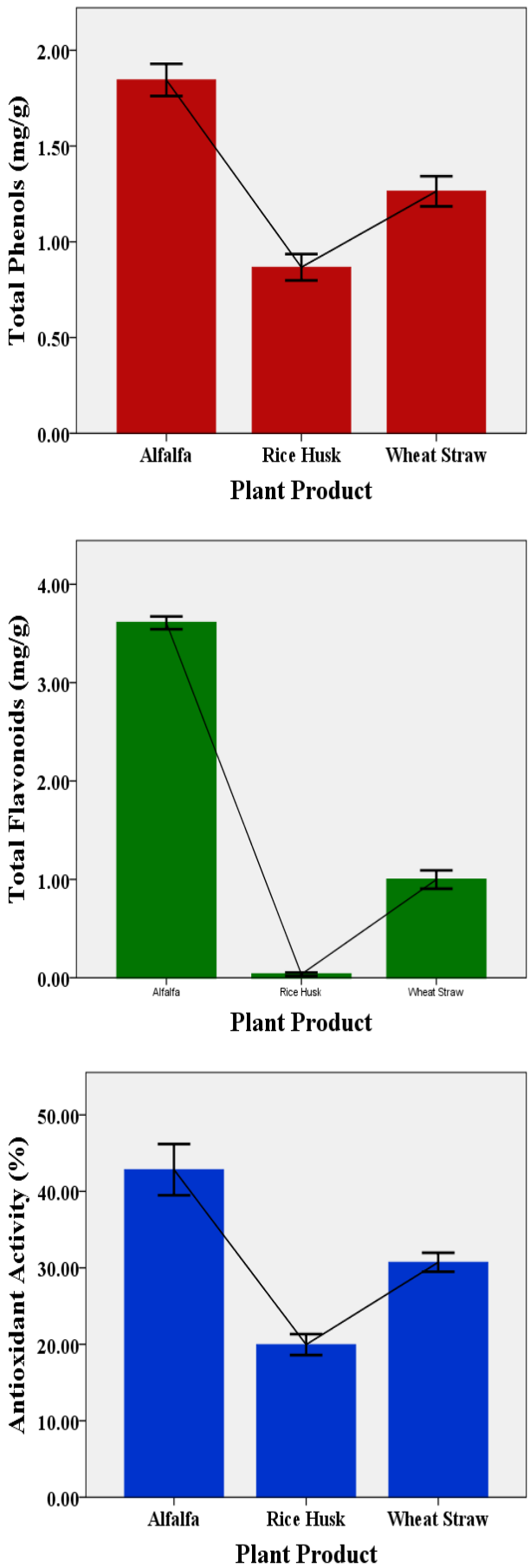

Fig. 1. Total Phenols (mg/g), total flavonoid (mg/g) and antioxidant activity (%) in different plant products.

Table 1. Inhibition percentage of phytopathogens by different plant product extracts using various solvents.

Pathogen	Treatment	Alfalfa	Rice husk	Wheat straw
Macrophomina phaseolina	Aqua	42.22 ± 2.22	41.48 ± 4.51	45.18 ± 0.74
	Methanol	85.18 ± 4.50	68.15 ± 3.23	75.55 ± 8.01
	Petroleum Ether	54.07 ± 0.74	57.78 ± 5.59	54.81 ± 9.46
	Cholroform	79.26 ± 10.45	60.00 ± 3.39	64.44 ± 3.39
Rhizoctonia solani	Aqua	40.00 ± 4.63	39.26 ± 3.23	38.52 ± 6.46
	Methanol	100.00 ± 0.00	96.30 ± 1.96	100.00 ± 0.00
	Petroleum Ether	82.22 ± 8.89	75.56 ± 2.22	97.04 ± 1.48
	Cholroform	91.11 ± 8.89	88.15 ± 11.85	65.93 ± 0.74
Fusarium oxysporum	Aqua	45.92 ± 3.92	45.93 ± 1.96	45.92 ± 2.67
	Methanol	96.30 ± 1.96	98.52 ± 1.48	100.00 ± 0.00
	Petroleum Ether	90.37 ± 9.63	89.63 ± 0.74	95.55 ± 2.22
	Cholroform	86.67 ± 8.41	100.00 ± 0.00	53.33 ±5.59
F. solani	Aqua	53.33 ± 2.57	45.93 ± 1.96	62.22 ± 7.80
	Methanol	98.52 ± 1.48	92.59 ± 4.51	97.78 ± 2.22
	Petroleum Ether	92.59 ± 1.48	79.26 ± 3.23	90.37 ± 5.19
	Cholroform	100.00 ± 0.00	85.19 ± 7.41	67.41 ± 5.34

Key note: ± = standard error of three replicates

Table 2. F-value and Significance level of total phenols (mg/g), total flavonoid (mg/g) and antioxidant activity (%) in different plant products.

Treatment	F-value	Sig. (*P*)
Total phenols	161.257	0.000***
Total flavonoids	3087.29	0.000***
Antioxidant activity	107.456	0.000***

Key note: Sig. (*P*) = Significance level: *p<0.05, **p<0.01 and ***p<0.001

Table 3. F-value and Significance level of inhibition percentage of phytopathogens by different plant product extracts using various solvents.

Treatment	Alfalfa		Rice husk		Wheat straw	
	F-value	Sig. (*P*)	F-value	Sig. (*P*)	F-value	Sig. (*P*)
Macrophomina phaseolina	12.34	0.002**	4.09	0.049*	6.80	0.014*
Rhizoctonia solani	15.73	0.001***	75.72	0.000***	15.90	0.001***
Fusarium oxysporum	11.52	0.003**	72.43	0.000***	394.35	0.000***
F. solani	177.74	0.000***	9.86	0.005**	19.00	0.001***

Key note: Sig. (*P*) = Significance level: *p<0.05, **p<0.01 and ***p<0.001

Discussion

Oxidative stress is a complex physiological and chemical mechanism in plant body that effectively led all abiotic and biotic stresses in plants which consequently results in the development of overproduction and accumulation of reactive oxygen species (ROS). The oxidative compounds are also responsible to cause the rottenness of food products due to which the nutritional profile and physical quality of fruits and vegetables are decreased. Therefore, antioxidant compounds protect plant cells from these damages by reacting against oxidative compounds (Pokorny *et al*., 2001). As some scientists stated that flavonoids and phenolic contents scavenge active oxygen and free radicals to protect the plant body from destruction by oxidative stress via complex formation and hydrogenation with particular oxidizing species (Sharma *et al*., 2009; Khalaf *et al*., 2008). According to Durmaz & Alpaslan (2007), highest activity of antioxidant compounds in plant is due to the greater amount of phenolic contents as antioxidant activity is stimulated by the quantity of phenolic contents. Our results also showed similar measures with this statement. The flavonoids and phenolic contents are the natural substances existing in different parts of the plant body that exhibit strong antioxidant activities against various environmental stresses. These compounds possess characteristics to receive an electron from ROS to form relatively more stable phenoxyl radicals. Therefore, the existence of flavonoids and phenolic contents may destroy the chain reactions of ROS in cellular functions in order to protect the plant body from harms (Pandey & Rizvi, 2009). In present studies, alfalfa and wheat straw have been found to contain highest amount of total phenols and flavonoids. Both plant products revealed highest activity of antioxidant by scavenging free radicals

of DPPH than rice husk as shown in Fig. 1. Thus, antioxidants have chief contribution with phenolic contents as they protect lipid peroxidation in fruits and vegetables. So, highest contents of flavonoids and phenols in plant body can enhance the ability of antioxidants against free radicals. Consequently, it is crucial to evaluate the amount of these substances in plant body for defense mechanisms. Hence, extracted phytochemicals from plant products revealed several defense activities such as antifungal, antibacterial etc. As Kodera *et al*., (2002) stated that phytochemicals have antifungal and antibacterial properties that rapidly produce at the infection part of plant body. Because these phytochemicals produces antioxidant substances to protect plant cells from injuries by reacting against oxidative compounds (Pokorny *et al*., 2001). According to several scientists, phytochemicals from different plants and their parts can be formulated as fungicides and may direct apply in the form of powder, cake, plant exudates or as extracts (Owino & Wando, 1992; Anjorin & Salako, 2009). Several scientists have been studied antifungal properties of phytochemicals from various plants whereas many researchers are still engaged in investigating innovative substances from plants (Juglal *et al*., 2002; Onyeagba *et al*., 2004; Boyraz & Ozcan, 2005; Benharref & Jana, 2006; Satish *et al*., 2007). Numerous edible plant extracts have been reported to comprise antifungal properties (Ferhout *et al*., 1999; Pradeep *et al*., 2003). Afzal *et al*., (2010) found that extract of *Allium sativum* contain a wide spectrum of antifungal activity that extended to 60-82% inhibition in mycelial growth of *Aspergillus* and *Penicillium*. Present studies also showed highest inhibition percentage by plant products extracting in methanol and chloroform that reached to significantly 100% inhibition of *M. phaseolina*, *R. solani*, *F. oxysporum* and *F. solani*. Similarly, *Piper betle* leaf extract have found to be effective against the growth of *A. flavus* and *Fusarium verticillioides* and it is reported that extract of 10,000 ppm completely inhibited its growth (Srichana *et al*., 2009). In another study, Hema *et al*., (2009) reported inhibition of fungal pathogens including *A. flavus* and other fungi by using herbs and South Indian spices. Among various extracts, 50% concentrated alcoholic extract of *Elettaria cardamomum* exhibited highest inhibitory activity against *Aspergillus niger*. Present study is agreed with the findings of Hema *et al*. (2009). It is revealed that methanol extracts of all plant products showed greater inhibition percentage against various phytopathogens as compared to other solvent extracts (Table 1).

Satish *et al*., (2007) examined aqueous extracts of fifty two plants belonged to various families against eight pathogenic species of *Aspergillus*. It was founded that twelve extracts among fifty two plants comprised significant antifungal properties for *Aspergillus* species. Similarly, antifungal properties of twenty two plant extracts were investigated by other scientists and reported that extracts of clove and ginger were found to be more effective among various plant extracts (Pundir & Jain, 2010). Adegoke & Odesola (1996) stated that extract of *Cymbopogon citratus* terminated the mycelial growth of pathogenic fungi such as *A. flavus* and *A. fumigatus*. Leaf powder of *Ocimum gratissimum* and *Syzygium aromaticum* with the mixture of packaging materials were found to be significantly effective against the disease of groundnut kernels caused by *A. parasiticus* (Awuah & Ellis, 2002). Large-scale consumption of phytochemical from various plants including azadirachtin from *Azadirachta indica* (Devkumar & Sukhdev, 1993), carvone from *Carum carvi* (Hartmans *et al*., 1995), allyl isothiocyanate from *Brassica* sp. and *Armoracia rusticana* oil (Ward *et al*., 1998) and eugenol by *Syzygium aromaticum* (Rana *et al*., 2011) attracted the attention of pathologists to discoveries of the new substances from plants against pathogens. Correspondingly, present findings also revealed greater amounts of phytochemicals and antioxidant properties in plant products that found to be successful in inhibiting various phytopathogens. These plant substances are bio-control agent that can be used to eradicate phytopathogenic soil-borne pathogens in the field of agriculture where they cause serious diseases in economically important crops (Shah *et al*., 2020). Moreover, these bio-control agents are eco-friendly as they are decomposable, renewable and harmless to animal and human health (Varma & Dubey, 1999).

Conclusion

Plants comprised several phytochemicals with various measures that are responsible for their defense mechanism against pathogenic growth. Highest amount of phyto-chemicals in plant product increased its ability to eradicate the mycelial growth of soil-borne phytopathogens like *M. phaseolina*, *R. solani*, *F. oxysporum* and *F. solani*. Consequently, extracted phyto-chemicals from plant products can be used to protect chief crops from various pathogenic disease in the field of agriculture.

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(Received for publication 12 October 2021)