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ANTIFUNGAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST SOIL-BORNE PHYTOPATHOGENS

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Abstract

In the present study, the extracts of different plant parts were used to control the growth of soil-borne fungi *viz.*, *Fusarium oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani*. Five plants including *Amaranthus viridis, Euphorbia hirta, Chenopodium album, Solanum nigrum* and *Carica papaya* were selected to check their antifungal and inhibition activity against soil-borne phytopathogens. The different parts of plants such as leaves, stems and fruits were dried and grounded for powder extract. The powder extract of three different concentrations 1, 3 and 5% were prepared and used against the soil-borne fungi by disc diffusion and food poison methods. Almost all plants showed antifungal activity against the *F. oxysporum, M. phaseolina* and *R. solani. A. viridis* and *E. hirta* showed maximum inhibition potential activity in disc diffusion method against above mentioned soil-borne pathogens as compared to other three plants, therefore *A. viridis* and *E. hirta* can be utilized for antifungal management of fungal diseases, particularly *F. oxysporum, M. phaseolina* and *R. solani.*

Key words: Antifungal activity, Soil-borne fungi, Plants extract, Inhibition.

Introduction

Bioactive chemicals obtained from natural sources like plants could be a potential source of antifungal, particularly in the present situation in which human and plant parasitic pathogens have embraced resistance against antifungal agents (Krishna *et al.*, 2008). Numbers of studies have been conducted to discover potential and environmentally safe techniques to control plant diseases (Agbenin *et al.*, 2004). The utilization of plant extracts for the controls of *Fusarium* wither in harvests is restricted (Agbenin & Marley, 2006). Tests done by Kimaru *et al.* (2004) uncovered that neem cake powder contains natural suppressing properties that have fungi static impacts against *Fusarium* wither of tomatoes. Seed extracts have antimicrobial activity with prominent effects on some fungal phytopathogens by Coventry & Allan (2001). Certain phytochemicals have potential to prevent mycelial growth of the soil-borne fungi which cause different diseases in plants (Ubulom *et al.*, 2011). All these plants contain minerals, essential metabolites-starches, amino-acids, non-polar constituents, proteins, fragrant cytokinins, hormones, auxiliary metabolites-flavonoids, saponins, terpenes, sterols, alkaloids and vitamins which exhibit the antifungal properties (Maiyo *et al*., 2010; Huang *et al*., 2012; Jabeen *et al.*, 2014b).

Agricultural chemicals are commonly used for management of pests and diseases. However, their use is costly as well as environmentally undesirable (Song & Goodman, 2001). Most of the chemical antifungal agents were used from the ancient times but they had many hazardous effects on environment as well as on humans (Tian *et al.*, 2012). Phytochemicals are non-nutritive plant chemicals that have defensive or illness preventive properties as compared to synthetic antifungal chemicals (Hussain *et al.*, 2014).

Papaya contains many secondary metabolites that have antifungal potency (Srinivasan *et al*., 2001). All the parts of papaya have different phytochemical concentrations and contain enzyme papain. Alkanoids are important group it shows defense mechanism against phytopathogens (Oliva *et al.*, 2003). The leaves of *Amaranthus viridis*, *Carica papaya* and *Solanum nigrum* showed significant inhibition against stem rot diseases (Chakrabarty *et al.*, 2014). *Chenopodium album* also revealed antagonistic effect against *M. phaseolina* (Banaras & Javaid, 2009; Javaid & Amin, 2009; Jabeen *et al.*, 2014a; Jabeen *et al.*, 2014b; Javaid & Iqbal, 2014). Among all soil-borne plant parasitic fungi the most important fungi are the *F. oxysporum, R. solani, M. phaseolina* (Wedgwood *et al.*, 2016). *Fusarium* species can damage the plant foliar as well as underground parts by abiogenetic spores (Agrios, 2005; Cuomo *et al.*, 2007). *Fusarium* species have numerous structures and morphology that exist as plant pathogens cause wilting and stem rot in banana, cotton, sweet potato, tomato, asparagus, muskmelon and cantaloupe (Ma *et al.*, 2010). *M. phaseolina* a soil-borne parasite causes charcoal rot in various plants. The organism can infect the root and lower stem of more than 500 plant species (Sinclair & Backman, 1993). The main objective of present study was a) design to set up different alternative method of inhibiting the growth of soil borne pathogens, and b) to minimize the use of different chemical controlling methods of pathogen.

Materials and Methods

Collection of plants: Five plant species including *Amaranthus viridis, Chenopodium album, Solanum nigrum, Carica papaya* and *Euphorbia hirta* were collected from various places including public parks, open fields, street/road verge and cultivated fields of Federal Urdu University of Arts, Science and Technology, Karachi (Table 1). The collected specimens were kept in polythene bags and labeled. The samples were transferred to the Dr. Abdul Ghaffar Lab of Aerobiology and Plant Pathology, Department of Botany, FUUAST, Karachi and kept at room temperature before *in vitro* screening. The specimen was dried at 40°C in oven and ground in an electric blender into powder, kept in polythene bags at room temperature until utilized (Zaidan *et al.,* 2005).

Preparation of pure culture of soil-borne fungi: The culture of *Rhizoctonia solani, Fusarium oxysporum, Macrophomina phaseolina* were collected from the mycobank of Dr. Abdul Ghaffar Lab of Aerobiology and Plant Pathology, FUUAST, Karachi and isolated by means of cork borer by the preparation of PDA (Potato Dextrose Agar), the disc of fungi was placed in the centre of the PDA poured plate under the sterilized conditions in safety chamber. Then the PDA plates were placed in the incubator for the proper growth.

Extract preparation: Five, three and one gram of plant's parts like flowers, stems and leaves of *Amaranthus viridis, Chenopodium album, Solanum nigrum, Carica papaya* and *Euphorbia hirta* were dried, then independently blended with 100 ml distilled water. The blend was allowed to stand for 24 hours and separated through a Whatman filter paper.

Disc diffusion method: The antifungal activity was evaluated by using of these plant extracts. The filter paper disc, (5mm in diameter) were prepared, sterilized and soaked in the plant extract of different concentration (1, 3, 5%) for 24 hours. These discs were placed in already prepared PDA plates and the fungal pathogen was placed is in the centre in sterilized conditions, then incubated and the radial growth was measured on the daily basis (Zaidan *et al*., 2005; Keyvan *et al*, 2009).

Poison food technique: Antifungal property is assisted by poison food technique (Nene & Thapliyal, 2000). Fungal culture is punched with a sterile cork borer in aseptic conditions. The discs of fungi were placed in PDA poured plates. The agar plates have been set up by infusing with different concentrations of plant extracts and incubated at temperature 28±1°C for each fungus. Diameter is recorded by measuring the two inverse outline of the fungal colony development. Rate of inhibition of mycelial development is assessed by calculating the inhibition percentage of diameter was noted by the formula (Verma *et al.,* 2011). The anti-fungal activity is calculated by the formula (Hussain *et al.,* 2015).

$$\text{Percent Inhibition} = \frac{\text{Y} - \text{Z}}{\text{Y}} \times 10$$

where Y = Alone pathogen (Mycelial growth) or control, Z = Growth activity of pathogen in the extract (Mycelial growth)

Statistical analysis of data: The statistical analysis of inhibition data and calculations of mycelial growth of phytopathogenic diseases were tabulated to one-way ANOVA (Analysis of variance) and Fisher's least significant test (LSD) at P=0.05 with Duncan's multiple range test (Gomez & Gomez, 1984).

Table 1. List of selected medicinal plants used for study.

Name of plant	Family	Local name	Part used	Uses
Amaranthus viridis	Amaranthaceae	Cholai	Leaf, stem, fruit	*Amaranthus viridis* is used as a medicinal herb in traditional Ayurvedic medicine
Chenopodium album	Chenopodiaceae	Bhatua	Leaf, stem	*Chenopodium album* has some medicinal properties like anthelmintic, antiphlogistic, antirheumatic, contraceptive, laxative, odontalgic etc. *Chenopodium album* used in the treatment of rheumatism, bug bites, sunstroke, urinary problems, skin problems, and to soothe burns
Solanum nigrum	Solanaceae	Mako	Leaf, stem	Infusions are used in dysentery, stomach complaints, and fever. The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a tonic, laxative, appetite stimulant, and for treating asthma and "excessive thirst". Traditionally the plant was used to treat tuberculosis
Carica papaya	Caricaceae	Papita	Leaf, fruit	Papaya is being investigated as an alternative to standard treatments for a range of ailments. *C. papaya* has a wide range of purported medicinal properties for treatment of diabetes, as birth control, as an antiseptic, antimicrobial, or diuretic, to control parasites, reduce inflammation, lower blood pressure, and lower cholesterol. While there are only limited data to support most of these uses, there is some evidence for healing bed sores and other wounds and in treating intestinal worms in human beings
Euphorbia hirta	Euphorbiaceae	Laldodhak	Leaf, stem, fruit	*Euphorbia hirta* is often used traditionally for feminine disorders, respiratory ailments (cough, coryza, bronchitis, and asthma) and worm infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems and tumors

Results

Antifungal activity of five plants was screened out by disc diffusion and food poisoning method against three soil-borne phytopathogens. In disc diffusion method, all plants show variant antifungal activity against selected three soil-borne phytopathogens. Maximum 82 and 80% inhibition was noted in leaf extract of *E. hirta* @ 5% concentration against the mycelia growth of *F. oxysporum* and *M. phaseolina,* respectively as compared to other remaining extracts and concentrations. As in the above results, the mycelial growth of the selected fungal soil-borne phytopathogens were less inhibited by leaf and stem extract *Chenopodium album* and *Solanum nigrum* (Fig. 1).

The results of food poisoning method were prominent and significant. The maximum 80% inhibition was observed in fruit extract of *Carica papaya* @ 5% concentration against *M. phaseolina*. However, the similarly *Chenopodium album* also showed 80% anti-fungal efficacy against *F. oxysporum*. While *R. solani* was mostly inhibited by *Carica papaya* as compared to other extracts (Fig. 2).

In ANOVA results of disc diffusion method showed that almost all factors showed significant ($p<0.001$) effects of leaf, stem and fruit against selected soil-borne fungal phytopathogens. Interaction of concentration and days did not show significant effect ($p<0.05$) of plant extracts of *E. hirta*, *S. nigrum* and *C. papaya* but found significant results in plant extract of *A. viridis*. The interaction between concentration and fungi showed non-significant ($p<0.05$) effects with respect to all variables. Interaction b/w days and fungi also showed non-significant ($p<0.05$) effect on extract of *E. hirta, C. album, S. nigrum* and *C. papaya*. The interaction among all three factors (Concentration× days× fungi) in disc diffusion was found non-significant ($p<0.05$) results almost in all variables. However, the ANOVA) results of food poison method, all factors including concentration, days and fungi species showed highly significant ($p<0.001$) effects of leaf, stem and fruit extract of *A. viridis, E. hirta, C. album, S. nigrum* and *C. papaya*. The interaction among concentration×fungi, days×fungi and the interaction of all three factors (Concentration× days× fungi) showed highly significant ($p<0.001$) effect on extract of *A. viridis, E. hirta, C. album, S. nigrum* and *C. papaya* and there was no any non-significant ($p<0.05$) effect were observed in any variable.

Discussion

Our study discloses that the extract of plant parts has potential to hinder the mycelial growth which revealed that these commonly available plants can also be used to control the dissemination and preventing the diseases. Plants deliver a high assorted qualities with an outstanding capacity against microbial pathogens on the premise of their harmful nature and repellence to microorganisms (Schafer *et al.,* 2012).The indole Acetic Acid (IAA) plays a vital role in plant growth and it also work in plant as a bio-control agent which hindered the spore germination and mycelial growth (Brown & Hamilton, 1993). Rauf & Javaid (2013) reported that ethyl acetate fraction of methanolic inflorescence extract of *C. album* has the potential to control *F. oxysporum*. In several studies, *C. album* also showed antagonistic effect against *M. phaseolina* (Javaid & Amin, 2009; Banaras & Javaid 2009; Jabeen *et al.,* 2014a; Jabeen *et al.,* 2014b; Javaid & Iqbal, 2014).

The plant *C. papaya* also contains many secondary metabolites that have antifungal potency (Srinivasan *et al.,* 2001). All the plants parts of *C. papaya* have different phytochemical concentrations and contain enzyme papain. Alkanoids of *C. papaya* is important group it shows defense mechanism again phytopathogens (Oliva *et al.,* 2003). *A. viridis, S. nigrum* and *E. hirta* are reported to inhibit the soil-borne fungi *Fusarium spp* (Gupta *et al.,* 2015). The plant extracts of *E. hirta* and *A. viridis* has been already investigated as antimicrobial and antifungal activities by Sudhakar *et al*., (2006) and Mallavadhani & Nasasimhan (2009). *A. viridis* treatments showed intermediate growth inhibition against soil-borne phytopathogens reported by Farooq *et al*., (2015). The leaves of *A. viridis*, *C. papaya* and *S. nigrum* showed inhibition against phytopathogen diseases (Chakrabarty *et al.,* 2014). According to Khan *et al*., (2018) that *S. nigrum* have strong suppressive potential against fungal growth of soil-borne phytopathogenic fungi including *M. phaseolina, F. oxysporum* and *R. solani*.

E. hirta has been proven to have wide pharmacological activities like antifungal, antibacterial and antinematicidal (Lanhers *et al.,* 1990; 1991). It shows significant inhibitions among all the plants against all the fungal pathogen. Since the 1980s, the antimicrobial acivities of *E. hirta* and its parts has been exhaustively explored by several researchers (Mallavadhani & Nasasimhan, 2009; Huang *et al*., 2012; Chakrabarty *et al.,* 2014). Bhaskara *et al*., (2010) has reported in his recent study that antifungal activity of leaves of *E. hirta* is more affective as compare to other parts of *E. hirta*. The different parts of *E. hirta* and *A. viridis* alongside its pharmacological activity have been considered by different researchers. The currents results support the earlier findings which demonstrate the presence of antimicrobial activity in different parts of *E. hirta* and *A. viridis* has been reported (Lipkin *et al.,* 2004; Cai *et al.,* 2005). The current results prove that selected plant extracts were effective and suppressed the radial mycelia growth of soil-borne phytopathogens and these findings confirm the result of Lipkin *et al*., (2004); Huang *et al*. (2012); Bhaskara *et al*., (2010) and Chakrabarty *et al*., (2014).

Conclusion

Almost all selected plant extract showed antifungal activity against soil-borne fungal phytopathogens. The utmost antagonistic effect was observed in highest concentration which is 5% in Disc diffusion method. *Amaranthus viridis* has shown maximum inhibition potential as compared to other plants. Mycelial growth in all the experiments were observed but minimum inhibition were found in *Euphorbia hirta* in Food poison technique while the maximum 83% restraining potency was observed in Papaya fruit which inhibit the mycelia growth of M. *phaseolina*as. In contrast to other extract of plant's parts leaf extract showed the maximal inhibition. It contains more phytochemicals which inhibits the fungi growth. The fruit and leaf of Papaya plant showed uttermost inhibition as compared to other two fruit extract.

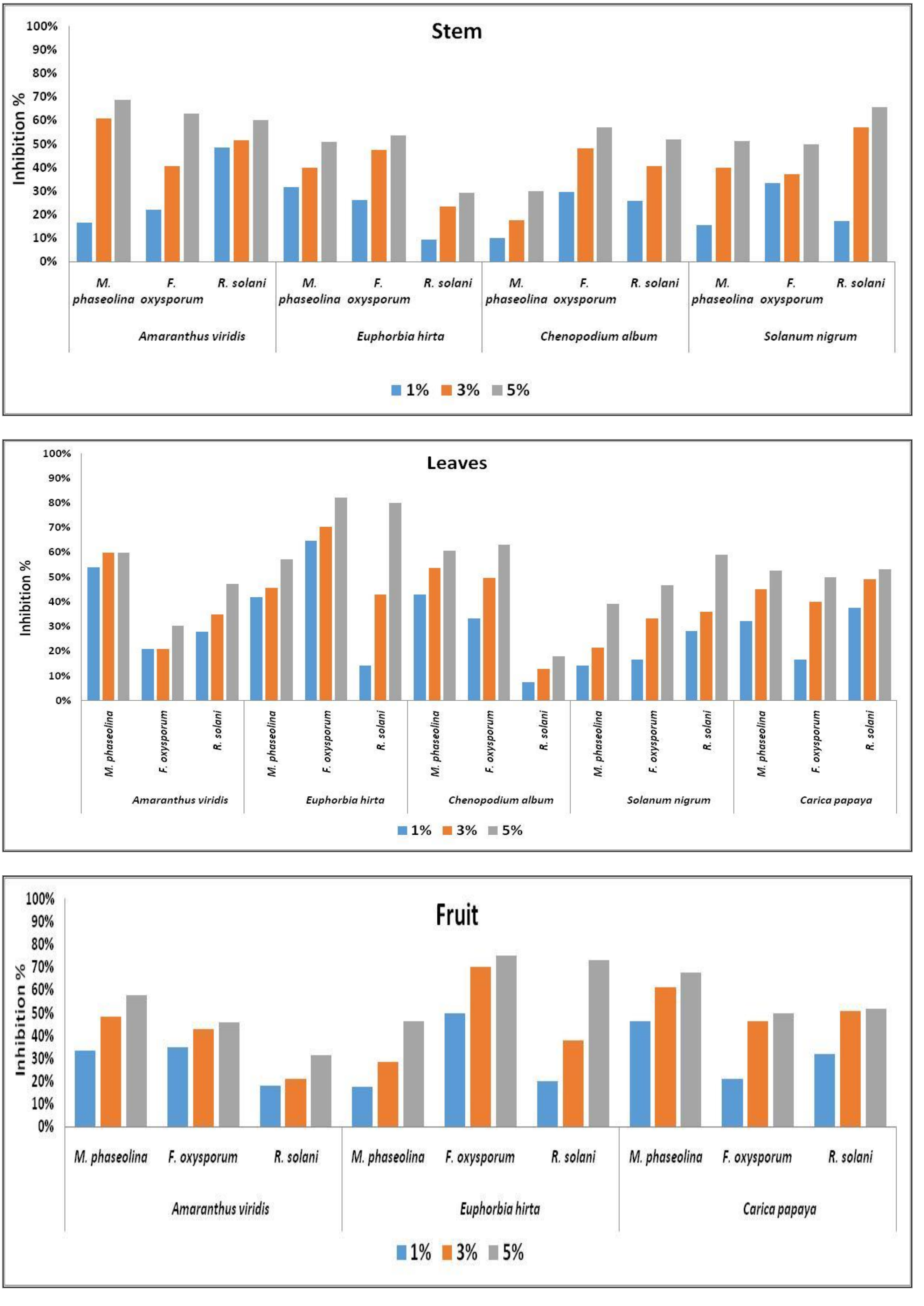

Fig. 1. Inhibition % of different plant parts (stem, leaves & fruit) against soil-borne pathogens in disc diffusion method.

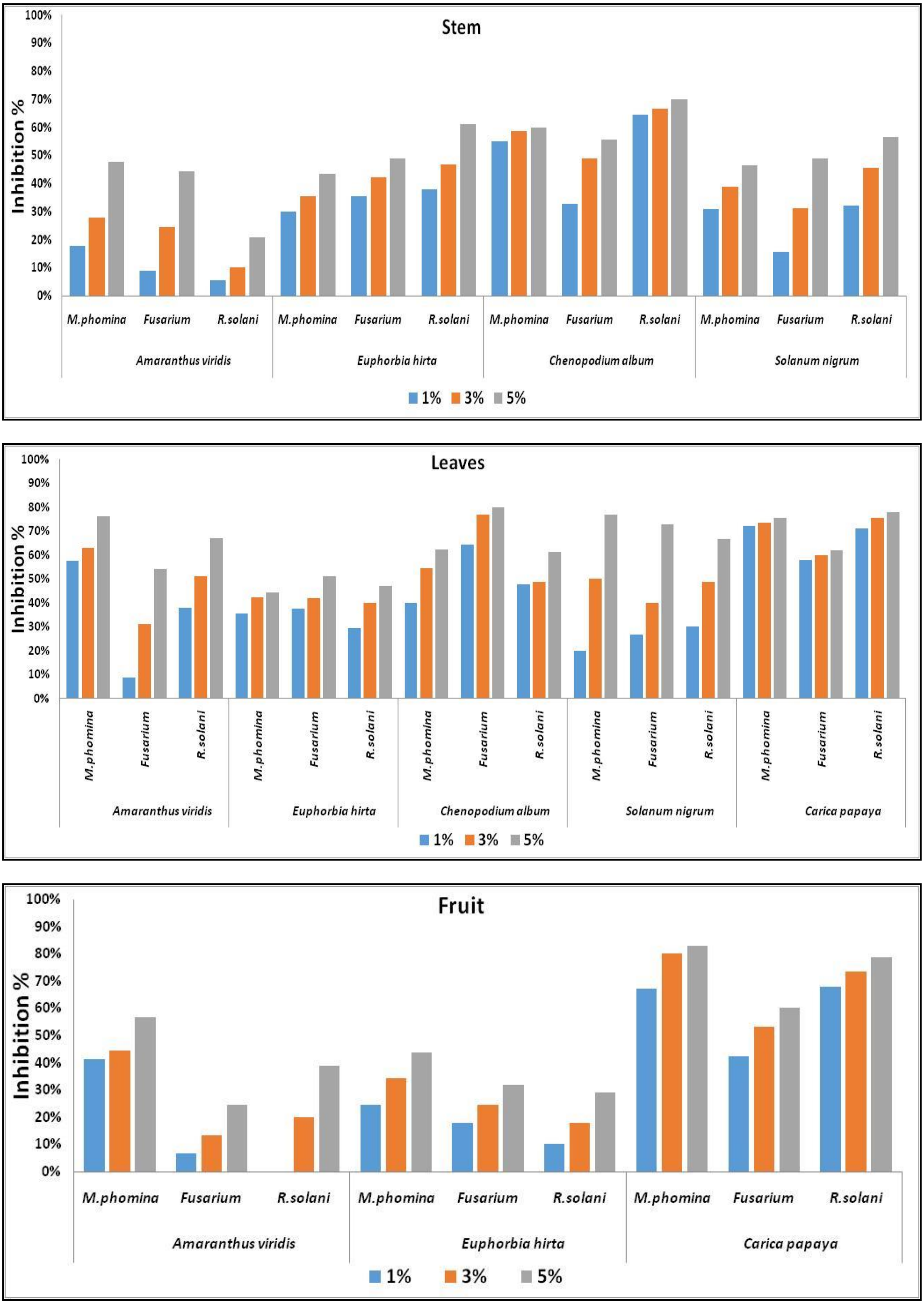

Fig. 2. Showing inhibition % of different plant parts (stem, leaves & fruit) against soil-borne pathogens in food poisoning method.

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