

# Identification and Assessment of Fungal Diseases of Major Medicinal Plants

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Abstract

Medicinal plants are infected by fungal diseases. A four years survey indicated that, there are various fungal infections in roots and shoots of several medicinal plants that grown in Isfahan. Lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*) and viper's bugloss (*Borago officinalis*) plants were infected by *Fusarium oxysporum* and *F. solani* on roots, showing wilting and eventually plants death, though, *F. culmorum* was also isolated, but without pathogenicity on these hosts. The sage plants (*Salvia officinalis*) were also infected by *F. solani* considerably. Burdock plants (*Arctium lappal*) were infected not only by *F. oxysporum* and *F. solani*, but also *Verticillium dahliae* and *V. albo-atrum* and common balm (*Melissa officinalis*) by *F. solani*. They were also simultaneously infected by *Rhizoctonia solani*. The aerial infections were mainly powdery mildews, downy mildews and rust. The naked seed pumpkin (*Cucurbita pepo* var. *sterica*) and common pumpkin (*Cucurbita pepo*) were infected by *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*., Johns-worth hypericum (*Hypericum perforatum*), by *Leveillula guttiferatum* and *E. hypersici*., estragon (*Artemisia dracunculus*) by *E. artemisiae*., bitter sweet (*Solanum dulcamara*) by *E. beceleate*., flixweld (*Descurainia sophia*) by *E. communiis*., marsh mallows (*Althaea officinalis* and *Malva silvestris*) by *L. malvacearum*., licoric (*Glycyrrhiza globra*) by *L. leguminosar*, dill (*Anethum graveolens*) and coriander (*Coriandrum sativum*) by *L. umbelleferatum*., downy mildews were observed on spinach (*Spinacia oleracea*) *Peronospora farinosa*, summer savory (*Satureia hortensis*) *P. Lamii* and waybread (*Plantago major*) *P. alta*. There was also rust diseases on medicinal plants including, estragon (*Puccinia absinthi*), wild thyme (*Thymus serpyllum*) *P. serpylli*, *P. mentha* and also on pudding grass (*Mentha pulegium*) and peppermint (*Mentha piperita*) *P. mentha*. white rusts, *Albugo candida*, were also observed on some of the medicinal plants including, flixweld and mother's heart (*Capsella bursa-pastoris*). The two species of *Verticillium dahliae* and *V. albo-atrum* with lower frequency on some of the medicinal plants especially on lavender, rosemary, pudding gross, pepper mint, castor (*Ricinus communis*) was found and there were typical symptoms on pepper mint. *Rhizoctonia solani* is an another fungal infecting agents, causing dry canker on roots and underground stems of some of the medicinal plants in this area.

**Keywords:** Downy mildews, Medicinal plants, Powdery mildews, Rusts, Root rots.

## INTRODUCTION

Medicinal plants were planted in Iran for a long time. Avicenna wrote many books about medicinal plants in Persian. As far as we know there are not comprehensive researches on wilt diseases and root rot of medicinal plants in Iran. Only damping off by *Phytophthora nicotianae* was reported from Castor (*Ricinus communis*) and *Fusarium oxysporum* was isolated from Cumin seed (*Cuminum cyminum*) (Ershad, 1996). In current years, there are some reports on fungal diseases in medicinal plants, but they are not complementary. Powdery mildews, *Erysiphe* and *Levillula* genera, was reported from different region of Iran. *E. sordido* was reported from way-bread (*Plantago major*) and Indian plantago seed (*Plantago psyllium*). *E. graminis*, *E. bicellata*, *E. artemisiae*, *E. communis*, *L. malvacearum*, *L. compositarum*, *Sphaerotheca fuliginea*, *L. taurica* and *L. leguminosarum* were reported from maidenhair fern (*Adiantum capillus veneris*), bitter sweet (*Solanum dulcamara*), estragon (*Artemisia dracunculus*), flixweld (*Descurainia sophia*), marsh mallows (*Althaea officinalis* and *Malva silvestris*), yarrow (*Achillea millefolium*), dill (*Aniethum graveolens*), coriander (*Coriandrum sativum*) and licorice (*Glycyrrhiza globra*), respectively. Also, *E. hyperici* and *L. guttiferarum* was reported from johns-worth hypericum (*Hypericum perforatum*) (Ershad, 1996). Moreover, leaf spot diseases, *Cercospora* and *Septoria* genera, were reported in some medicinal plants. *C. althaeina*, *C. ricinella*, *S. rubiae*, *S. rechingeri*, *S. sisymbrii* were isolated from marsh mallows, castor, madder (*Rubia tinctorum*), currant fruited rhubarb (*Rheum ribes*) and flixweld, respectively. Downy mildew caused by *Pernospora* genus, this genus including *P. farinose*, *P. alta* and *P. lamii* were isolated from spinach (*Spinacia oleracea*), way-bread and summer savory (*Satureia hortensis*), respectively (Ershad, 1996). Also, rust disease including *Puccinia* and *Uromyces* genera were found in medicinal plants. *P. menthae* and *P. serhylli* were found in wild thyme (*Thymus serpyllum*). *P. achilpeae*, *P. graminis*, *P. menthe*, *P. malvacearum*, *P. absinthi*, *P. dracunculine*, *U. glycyrrhizae* were found in yarrow, maidenhair fern, peppermint (*Mentha piperita*) and pudding grass (*Mentha pulegium*), marsh mallows, estragon, madder, licoric, respectively. Loose smut, *Ustilago nuda* was reported in maidenhair fern, white rust, *Albugo candidates* was found in flixweld and mother's heart (*Capsella bursa-pastoris*) (Ershad, 1996).

Fungal diseases also were reported on medicinal plants around the world. *Rhizoctonia solani* was identified as a leaf spot disease in malabar nut (*Adhatoda vasica*) in India (Verma *et al.*, 2006). *Pithomyces chartarum* is known to cause leaf spot diseases of ashwagandha (*Withania somnifera*) in India (Verma *et al.*, 2007). Wilt disease of cucumber (*Cucumis sativus*) caused by *F. oxysporum* f. sp. *cucumerinum* has been recorded in Turkey for a long time (Yıldız and Delen, 1977). Also *F. oxysporum* f. sp. *radicis-cucumerinum* causes wilting accompanied by root and stem rot has been reported in this country (Karaca and Kahveci, 2009) and in British Columbia (Punja and Parker, 2000). *Macrophomina phaseolina* was found to cause root rot in medicinal coleus (*Coleus forskohlii*) in India (Kamalakkannan *et al.*, 2005). *Peronospora lamii* causing damage to sage (*Salvia officinalis*) and rosemary (*R. officinalis*) reported from the UK (Humphreys-Jones *et al.*, 2006). *Fusarium* wilt caused by *Fusarium solani* on commercial field lavender was identified in China (Ren *et al.*, 2007). Several species of powdery mildew fungi have been recorded on rosemary (*Levillula* spp.) from Europe and *Podosphaera fuliginea* from USA (Farr and Rossman, 2009). Powdery mildew on rosemary associated with *Golovinomyces biocellatus* in Asia (Park *et al.*, 2009). *Podosphaera fusca* (syn. *Sphaerotheca fusca* and *S. fuliginea*) has been recorded to infect German chamomile (*Matricaria chamomilla*) in Canada, Egypt, Germany, Switzerland, Russia (Farr and Rossman, 2009). *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum*) is a rather common powdery mildew species infecting German chamomile in Europe (Farr and Rossman, 2009) and has been reported in Korea (Park *et al.*, 2010).

There is no any report on vascular wilt disease, root rot and plant death on medicinal plants on the medicinal plants so far. Only damping-off disease caused by *Phytophthora nicotianae* and *Fusarium oxysporum* were deducted from castor and cumin seed (*Cuminum cyminum*), respectively

(Ershad, 1996). This report is dealing with the fungal diseases, occurring on medicinal plants in Isfahan Province, Iran.

## MATERIALS AND METHODS

The surveys on fungal diseases were carried out as a national project in medicinal plants for 4 continuous years (2005-2008). For this purpose, the fields of medicinal plants were surveyed throughout early years and samples of plants that showed symptoms of wilting, damping off and leaf spots were collected.

The number of healthy and infected plants were recorded and then registered in tables, separately. All the samples with the symptoms of disease were collected in the separate plastic bags. The name of plant, place and time of samplings were recorded accordingly. Samples were taken into the lab as soon as possible in order to examination in due course time stored sample at refrigerator were used to isolation of fungal agents, and keep in the fridge (Anon, 1985).

For isolation of root rot fungal agents, samples were washed thoroughly in running tap water for 30 min to remove the debris adhered. Samples were carefully dissected into small pieces from border of healthy and infection regions of the roots. Then, surface-sterilized roots were placed on petri dishes containing potato dextrose agar medium (PDA) and incubated at  $25^{\circ}\text{C}\pm 1$  and 12h of illumination in incubator. Sub cultures were obtained from hyphal tips of the developing fungal colonies then were placed in incubator again.

Spore suspensions of fungal isolates were cultured on WA medium as a single colony, in order to obtain pure culture. Eventually, same single colonies were transferred randomly into the PDA medium in the same conditions as describe above. SNA medium was used for avoiding mutation in *Fusarium* spp. (Singleton *et al.*, 1993).

*Fusarium* genus was identified by CLA medium or clove leaf agar and also identified base on several keys and references (Barnett and Hunter, 1999). Petri dishes containing sterile and wet WhatMan paper were used to identify *Verticillium* genus. Hypha and spores of fungi were collected from solid medium and then fungal isolates with a spore concentration of about 10<sup>7</sup> spores per ml were propagated to prove pathogenicity tests. Inoculum of isolates were added to pots containing sterilized soil (soil: sand, 1:1ratio) (Booths, 1971).

The seedlings of the medicinal plants had been planted in sterilized pots and then were transferred into pots with infested soils. Eventually, pots were placed in greenhouse conditions for the further growth of tested medicinal plants (Joubert *et al.*, 1970; Nasr Esfahani, 2004).

Some groups of plant pathogenic agents, namely downy mildews, powdery mildews, rusts and smuts could not grow on most commonly used nutrient media in lab conditions. So, these fungi were identified as such, according to microscopic structures and classified on several related keys and references (Ershad, 1996; Barnett and Hunter, 1999; Farr and Rossman, 2009).

## Statistical Analyses

Variance analysis was summarized using statistical analysis system (SAS) version 8 (SAS Institute, Inc., Cary, NC). Duncan's test (DMRT) in the level of 1% was performed to determine the significance of differences in the means of infection percentage.

## RESULTS

Results indicated that, there are several fungal diseases, which may infect the medicinal plants in Iran out of which, there are on aerial and soil parts of the infected plant, depending upon the plant species and the fungal pathogens. Pathogenicity tests were carried out in greenhouse conditions and the fungi were re-isolated from these plants and identified as described below. The results are also summaries in table 1.

### **Rosemary (*Rosmarinus officinalis*)**

Wilting and die of the plant tissues in rosemary was occurred in Janatabad station. Infected plants began to show wilting in leaves and grow poorly. Eventually, death and collapse was observed in these plants. The infection rate of root rot and wilting was of 40.81%, which a considerable amount in comparison to other studied medicinal plants in these studies with a significant effect (Table 1). *F. oxysporum* and *F. solani* was known to cause disease in rosemary (Table 1), while *F. culmorum* was not the cause of the disease. So, this genus was in association with *F. oxysporum* and *F. solani*.

### **Lavander (*Lavandula angustifolia*)**

Decline rate in lavender plants was also considerable in comparison with other medicinal plants. Even, root and crown rot was observed in lavender plants, which was planted in public parks and landscape. Infection rate in these plants in Janatabad station was 20% with a significant effect. It was categories in a signal statist of group along with sage as far as the disease severity is concerned (Table 1). *F. solani* and *F. oxysporum* were isolated from this plant. These isolate was obtained on PDA medium and maintain on SNA medium. CLA medium was used to identify.

### **Sage (*Salvia officinalis*)**

Wilting and dryness also was found in sage plants like other medicinal plants. Infected bushes were shown green death and stem became black. Roots begin to be infected and then stem and crowns were infected by fungi. *F. solani* was isolated from infected sage plants. Number of infected and health plants were counted and infection rate was evaluated accordingly (Table 1).

### **Viper's bugloss (*Borago officinalis*)**

Wilt disease was the most prevalent disease in Janatabad station. All of perennial plants died after two years because of severe infection. When plants were replaced in this station in the same year, infection was observed in Viper's bugloss again (Table 1). *F. oxysporum* and *F. solani* were isolated from PDA medium and maintained in SNA. CLA medium was used to identify them.

### **Burdock (*Aractium lappal*)**

Wilt and dryness diseases also were observed in burdock bushes. The symptom was green dead in leaves, black rots in petiole and severe rots in stems and roots. Burdock plants had been infected not only by *Fusarium* species (*F. oxysporum*, *F. solani*) but also infected by *Verticillium dahlia* and *V. albo-atrum*. (fields have become contaminated with *Verticillium*). Wilt bushes were counted in order to evaluate infection rate in 4 random blocks (Table 1).

### **Common balm (*Melissa officinalis*)**

Dryness (chlorosis, necrosis) also was found in common balm plants in Janatabad station. Dry bushes were counted with 4 repeat to evaluate infection rate (Table 1). *F. solani* and *Rhizoctonia solani* were isolated and studied in lab and greenhouse.

### **Naked seed pumpkin (*Cucurbita pepo var. sterica*)**

Naked seed pumpkin had been infected by powdery mildew. On the leaves and stems, disease appears at first as small, white and powdery lesions that soon become covered with large, white patches of fungus growth. At the short time fungus cover both sides of leaves entirely. Eventually leaves become necrotic and died. The pathogens that cause powdery mildew on vegetables are introduced as *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*. *S. fuliginea* is more probable to cause diseases in vegetables (is probably the most common and widespread diseases on vegetable). The infection rate of naked seed was estimated 100% (Table 1).

### **Johns-worth/hypericum (*Hypericum perforatum*)**

This plant also was infected by powdery mildew. Symptoms on leaves were the same as symptoms on naked seed. *Erysiphe hyperici* and *Levillula guttiferarum* causes powdery mildew on Johns-worth/hypericum. White patches of fungus growth covered the entire surface of growing leaves and infection rate was assessed 100% (Table 1).

### **Other medicinal plants**

Evidences show that powdery mildews are the most common diseases on medicinal plants. The late in the end of season, september, powdery mildews were characterized by the appearance of spots on patches of a white, powdery, mildew growth on plant tissues and then entire leaves completely were covered by the white powdery mildew. *Sphaerotheca*, *Erysiphe* and *Levillula* were identified and introduce in references.

In blocks and fields powdery mildew diseases was observed on the vast number of medicinal plants including estragon *Artemisia dracuncululus* (*Erysiphe artemisiae*), bitter sweet *Solanum dulcamara* (*E. bicellate*), flixweld *Descurainia sophia* (*E. communis*), marsh mallows *Althaea officinalis* and *Malva silvestris* (*Levillula malvacearum*), licoric *Glycyrrhiza gloabra* (L.) *legumiosarum*, naked seed pumpkin *Cucurbita pepo* var. *sterica* and common pumpkin *Cucurbita popo* (*Sphaerotheca fuliginea*), Vipers bugloss *Borago officinalis* (*E. asperifoliarum*), dill *Anethum graveolens* and coriander *Coriandrum sativum* (*L. umbelliferarum*). Leaves of some medicinal plants were used for different purpose, so, powdery mildew is a significant disease on these medicinal plants.

The downy mildew of medicinal plants was the genus *Pernospora*. Downy mildew infected the spinach (*Spinacia oleracea*) very seriously. *P. farinose* was found as the disease agent. This disease also was observed on summer savory (*Satureia hortensis*) in the field and *P. lamii* cause disease on them. *P. alta* was reported as disease agent on waybread (*Plantago major*).

Plant rusts are among the most destructive plant disease on medicinal plants. *Puccinia* and *Uromyces* was identified and reported on medicinal plants in different references. Various species of *Puccinia* infected the medicinal plants and cause rust disease, including estragon *Artemisia dracuncululus* (*P. absinthi*), Wild thyme *Thymus serpyllum* (*P. mentha* and *P. serpylli*); causing severe infection on leaves and stems, pudding grass *Mentha pulegium* and peppermint *Mentha piperita* (*P. mentha*).

White rust also was observed on some medicinal plants including: Flixweld and mother's heart (*Capsella bursa-pastoris*). *Albugo candida* was introduced and identified on these plants.

Soil borne (some fungi are widespread in soils) disease are the common and significant disease on medicinal plants. *Verticillium dahliae* was found on the vast number of plants. These fungi grow on the medium very slowly and isolation of them is very difficult. On the most time, other fungi like saprophyte grow on the media with these fungi and so develop on media very late.

Nevertheless, the abundance of *V. dahliae* and *V. albo-atrum* was very low in vascular tissues of roots and stems of some medicinal plants especially annual and perennial plants. *Verticillium* was observed in some medicinal plants including lavender (*Lavandula angustifolia*), rosemary (*Rosmarinus officinalis*), pudding grass (*Mentha pulegium*), peppermint (*Mentha piperita*), Castor (*Ricinus communis*) and other plants, but the symptoms like wilt and death of the entire plant was not observed significantly and clearly in these plants. However, *Verticillium* infection results in describing growth of plants. Also, the symptoms of *Verticillium* wilt and chlorotic in some branches was found significantly in peppermint.

*Rhizoctonia* disease causes losses on most medicinal plants in this study. The most common symptoms caused by *Rhizoctonia* on most plants was root rot and then there was the appearance of stem canker on annual plants including castor (*Ricinus communis*), yarrow (*Achillea millefolium*), common balm (*Melissa officinalis*) and perennial plants including lavender (*Lavandula*

*angustifolia*), rosemary (*Rosmarinus officinalis*), marsh mallows (*Althaea officinalis* and *Malva silvestris*), viper's bugloss (*Borago officinalis*), burdock (*Aractium lappal*) and etc. *Rhizoctonia* also causes dry canker on underground stems (root stock, rhizome) and rotting of axillaries roots hair.

Physiological diseases also cause diseases in medicinal plants. Deficiency of elements was observed in most plants. Certain symptoms were chlorosis of leaves, scorching of the margins, parallel and symmetric discoloration of leaves. Common balm (*Melissa officinalis*) was sensitive to deficiency of iron and upper leaves turn white and discolored.

## DISCUSSION

Medicinal plants like other plants have special diseases. Data in this survey show that various fungi disease appears on medicinal plants and infected aerial, foliage and underground parts of plants. Powdery mildews are airborne disease. This disease appeared on leaves and fresh stems on the late of the season and then covered entire surface of growing parts of plants. The symptoms of powdery mildew had been reported already by other researcher.

All data was collected by Ershad and was published in fungi books in 1996. In our study, powdery mildew was reported on some medicinal plants in the first time including: Naked seed pumpkin (*Cucurbita pepo* var. *sterica*) and common pumpkin (*Cucurbita pepo*), Johns-worth hypericum (*Hypericum perforatum*), bitter sweet (*Solanum dulcamara*), flixweld (*Descurainia sophia*), marsh mallows (*Althaea officinalis* and *Malva silvestris*). This disease was already observed on estragon (*Artemisia dracunculus*), dill (*Aniethum graveolens*) and coriander (*Coriandrum sativum*) in Esfahan fields.

Another airborne disease is rust. This disease was observed on estragon (*Artemisia dracunculus*), wild thyme (*Thymus serpyllum*), pudding grass (*Mentha pulegium*) and peppermint (*Mentha piperita*). Also, other researchers have introduced rust disease on medicinal plants. In our study, these diseases were mentioned. White rust, airborne disease, was found on flixweld and mother's heart (*Capsella bursa-pastoris*). White rust has been reported in other regions of Iran, recently. So, control of airborne disease is very important because aerial parts of these plants are used for some medicinal purposes. So, every kind of control methods cannot be offered by researchers and or cannot be applied by farmers.

Therefore, it is necessary that other control methods will be offered by researchers like eugenics and cultural methods, integrated pest management to reduce chemical controls, using fungicide with low residue and then these methods should be performed as comprehensive national project. Control methods are very significant especially on medicinal plants which aerial parts of them and their leaves are used as medicinal purposes. Dryness, wilt and death of tissues were observed on medicinal plants by soil borne fungi. *F. oxysporum* and *F. solani* were two significant species and isolated from roots of medicinal plants. These two species was very common and widespread on rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula angustifolia*). Also, these plants were planted as ornamental plants on public parks and landscape. Damping off was so important in these regions. *F. oxysporum* only was reported and isolated on cumin seed (*Cuminum cyminum*) by other researchers (Ershad, 1996). Sage (*Salvia officinalis*), burdock (*Aractium lappal*), common balm (*Melissa officinalis*) and other plants were infected to Fusarium especially *F. solani* but the symptoms was not so clear and severe disease and infection was not found on other plants by Fusarium species.

Another soil borne fungi was *Verticillium*. *V. dahlia* and *V. albo-atrum* was identified and isolated from medicinal plants. The prominent species was *V. dahlia*. The frequency and pathogenicity of this species was higher other than *Verticillium* species. Also, *V. dahlia* produce microsclerote on infected tissues in wet conditions and it was observed by binocular microscope, easily. *Verticillium* genus was isolated from vascular tissues of roots and underground stems of me-

dicinal plants especially on rosemary (*Rosmarinus officinalis*), lavender (*Lavandula angustifolia*), pudding grass (*Mentha pulegium*), peppermint (*Mentha piperita*), castor (*Ricinus communis*) and etc. This fungus penetrates on vascular tissues systemically and then it transfer from stem to aerial parts of plants, easily. When stem of plant was cut and grow on wet chamber, *Verticillium* grow and develop on stem. It is necessary to mention that this fungus grow poorly and slowly on nutrient medium and isolated difficulty. Nevertheless, research and survey about *Verticillium* has not been done completely and significantly (Snowdon, 1991; Singh, 1992; Sutton, 1993). The symptoms of cancer, root rot, axillaries root rot of medicinal plants were caused by *Rhizoctonia solani*. This fungus cause canker on underground stems of medicinal plants, especially on thick underground stems. This disease cause scar and death of tissues and finally it cause medicinal plants to grow poorly.

This study show that root rot, vascular wilt, damping off of medicinal plant, which cause by soil borne fungi, are very significant disease in medicinal plants. Thus, it is probable medicinal plants will be planted in the vast number of fields. It is necessary that researcher consider and survey different kind of control methods or prevention of soil borne fungi disease. Moreover, control of pathogen agents of medicinal plants is different from other agricultural plants. In future, regarding to problems of chemical medicine, medicinal plants maybe will be used more than now and medicinal plant will be very important plant in future. So, it is a nice suggestion that research project concentrate on nonchemical methods or integrated pest management in order to decrease consuming of chemical and prevent adverse effects of them on human and nature (Walker, 1952; Candan and Suludere, 1999; Amponsah *et al.*, 2002; Merle shepard, 2003; Purohit and Vyas, 2004;).

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## Tables

Table 1. The infection of medicinal plants to fungal diseases.

No	Common name	Scientific name	Casual organisms	Mean No. of plants				Duncan's group
				Total /Rep	Healthy/Rep	Infected/Rep	Infection (%)	
1	Rosemary	<i>Rosmarinus officinalis</i>	<i>Fusarium oxysporum</i> <i>F. solani</i> <i>F. culmorum</i>	17.25	12.25	6.60	40.81	c
2	Lavender	<i>Lavandula angustifolia</i>	<i>F.oxysporum</i> <i>F. solani</i>	30.00	25.00	5.00	20.00	ab
3	Sage	<i>Salvia officinalis</i>	<i>F. solani</i>	22.75	18.25	4.50	24.65	ab
4	Viper's bugloss	<i>Borago officinalis</i>	<i>F.oxysporum</i> <i>F. solani</i>	22.75	16.00	6.75	42.18	bc
5	Burdock	<i>Aractium lappal</i>	<i>F.oxysporum</i> <i>F. solani</i> <i>Verticillium dahlia</i> <i>V. albo-atrum</i>	30.00	25.00	5.00	20.00	ab
6	Common balm	<i>Melissa officinalis</i>	<i>F. solani</i> <i>Rhizoctonia solani</i>	21.75	19.00	2.75	14.47	a
7	Naked seed pumpkin	<i>Cucurbita pepo var. sterica</i>	<i>Erysiphe cichoracearum</i> <i>Sphaerotheca fuliginea</i>	59.50	29.75	29.75	100.00	d
8	Johns-worth/hypericum	<i>Hypericum perforatum</i>	<i>E. hyperici</i> <i>Leveillula guttiferarum</i>	34.00	19.50	19.50	100.00	d

Means followed by the same letter are not significantly different at P=0.01 level in Duncan's multiple range test