Journal of Crop and Weed, 14(1): 224-229 (2018)

Phylloplane microflora diversity of rose and mycoparasitism over rose powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary)

V. KUMAR AND S. CHANDEL

Department of Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni-173230, Solan, Himachal Pradesh

Received : 10-01-2018 ; Revised : 15-03-2018 ; Accepted : 20-03-2018

ABSRACT

Phylloplane microflora diversity of rose was assessed during the winter and summer seasons in year 2015. Eight fungal flora i.e. Fusarium sp., Botrytis sp., Cladosporium sp., Penicillium sp., Aspergillus sp., Alternaria sp., Trichothecium sp., Trichoderma sp. and two bacterial isolates (Bacillus sp. and Pseudomonas sp.) were isolated from the phylloplane of the rose leaves in both seasons. The highest frequency of microflora occurrence in all months of summer and winter seasons were Fusarium, Aspergillus and Penicillium and bacterial isolates (Bacillus sp. and Pseudomonas sp.), while the Cladosporium sp. had observed only in the month of June. Further this isolated microflora was evaluated against the powdery mildew fungus under in vitro conditions by using slide spore germination test. The in vitro studies revealed that all the culture filtrate were effective against the rose powdery mildew. The highest conidial germination inhibition of 21.88 per cent was recorded from the culture filtrate of Trichoderma sp. followed by 16.20 per cent of Alternaria sp., Botrytis sp. (11.58%), Bacillus sp. (11.45%), Trichothecium sp. (10.89%) and Fusarium sp. (8.68%). The non effective culture filtrates with minimum conidial inhibition (2.66%) were Aspergillus sp. followed by Pseudomonas sp., (5.08%), Penicillium sp. (5.44%) and Cladosporium sp. (7.98%).

***Keywords*:** Microflora, phylloplane, powdery mildew, rose

Rose has been admired for its beauty and fragrance since its first cultivation 5000 years ago by ancient civilizations of China, Western Asia and Northern Africa (Gudin, 2000). After selection and breeding for thousand years, especially after the first Hybrid tea roses were bred, rose has become one of the most economically important ornamental crops. The genus *Rosa* comprises more than hundred botanical (wild) species, of which only about ten contributed to the development of cultivated roses: *R. chinensis*, *R. foetida*, *R. gallica*, *R. gigantean*, *R. moschata*, *R. multiflora*, *R. phoenicea*, *R. rugosa*, *R. wichurana* and *R. rubra* (Crespel and Mouchotte, 2003). Most of the roses grown today are not true species but are derivatives of interspecific hybridization (Zhang, 2003), leading to a wide diversity among cultivated roses.

The presence of natural antagonists of powdery mildew of rose from the phylloplane of rose leaves and other crops play important role as they utilize their antagonistic properties in disease management program.The most commonly associated antagonists with oak powdery mildew were *Acremonium* sp., *Trichoderma* sp., *Ampelomyces* sp., *Phoma* sp. and *Leptosphaerulina australis*. Nearly 90 per cent of mildew colonies were associated with *L. australis*, which is not generally considered as a mycoparasite or antagonist, in contrast with the other three fungi. *Acremonium* sp. abundance was greater in summer samplings, whereas *L. australis* and *Trichoderma* sp. predominant in autumn samplings. *Ampelomyces* sp. and *Phoma* sp. was never observed in the absence of powdery mildew (Topalidou and Shaw, 2015). Similarly *Acremonium* spp., *Ampelomyces* spp., *Penicillium* spp., *Cladosporium* spp., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., *Bradyrhizobium* spp., *Brachybacterium* spp., *Curtobacterium* spp., *Cryptococcus* spp., *Rhodosporidium* spp., and two unidentified yeasts were most effective phylloplane microflora against the powdery mildew of dogwood (Mmbaga *et al.*, 2008). *In vitro* maximum inhibitory effect was exhibited by *B. spicifera* followed by *C. lunata*, *C. cladosporioides*, *Alternaria Alternata* against *Erysiphe polygoni*. The percentage of disease inhibition was most significant with *C. cladosporioides* followed by *A. alternata*, *F. semitectum* and *A. niger* (Suman, 2008).

The knowledge of phylloplane microflora has been important for management of foliar diseases keeping in view of the adverse effect of fungicides on environment and run down of host resistance over a period of time. Indigenous fungal as well as bacterial populations are found efficient to suppress the diseases by reducing pathogen population and thereby minimizing the disease severity (Maji, 2003). In the present study was carried out to ascertain the frequency of occurrence and isolation of phylloplane microflora from the powdery mildew infected rose plants during summer and winter season. Efficacy of culture filtrate of isolated microflora from rose phylloplane against the rose powdery mildew was examined for its management.

Email: vnarwal777@yahoo.com

Table 1: Frequency of occurrence of phylloplane microflora of rose during summer and winter seasons (2015)

Microflora	Summer season			Winter season		
	April	May	June	October	November	December
Fungi						
Fusarium sp.	+	+	+	+	+	+
Botrytis sp.	-	-	+	+	+	-
Cladosprium sp.	-	-	+	-	-	-
Penicillium sp.	+	+	+	+	+	+
Aspergillus sp.	+	+	+	+	+	+
Alternaria sp.	+	+	-	+	-	-
Trichothecium sp.	+	+	+	+	-	-
Trichoderma sp.	-	+	+	-	-	-
Bacteria						
Isolate 1	+	+	+	+	+	+
Isolate 2	+	+	+	+	+	+

***+: Present, -: Absent**

Table 2 : *In vitro* evaluation of culture filtrate of isolated phylloplane microflora against *Podosphaera pannosa* causing powdery mildew of rose for antagonistic properties

Microflora	Conidial germination (%)	Conidial germination inhibition (%)
Fungi		
Fusarium sp.	52.60 (7.32)	8.68 (3.11)
Botrytis sp.	50.93 (7.21)	11.58 (3.55)
Cladosporium sp.	53.00 (7.35)	7.98 (2.99)
Penicillium sp.	54.47 (7.45)	5.44 (2.54)
Aspergillus sp.	56.07 (7.55)	2.66 (1.90)
Alternaria sp.	48.27 (7.02)	16.20 (4.15)
Trichothecium sp.	51.33 (7.23)	10.89 (3.44)
Trichoderma sp.	45.00 (6.78)	21.88 (4.78)
Bacteria		
Bacillus sp.	51.00 (7.21)	11.45 (3.53)
Pseudomonas sp.	54.67 (7.46)	5.08 (2.45)
Control	57.60 (7.66)	-
CD0.05	0.28	0.33

***Figures in parentheses are square root transformed values*

MATERIALS AND METHODS

Isolation of phylloplane microflora from powdery mildew infected rose leaves

Isolation of microflora were taken from powdery mildew infected rose plants by leaf wash method on potato dextrose agar medium, malt extract agar medium and nutrient agar medium in petriplates. Fungal cultures were purified by hyphal-tip method. Cultures of bacteria were maintained on PDA and nutrient broth agar slants respectively, under refrigerated conditions.

Preparation of the culture filtrates from microflora

Culture filtrates of different fungal isolates i.e. *Fusarium* sp., *Botrytis* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Trichothecium* sp., *Trichoderma* sp. and two bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.). The fungal isolates were cultured on potato dextrose broth (PDB) for 15 days at 20–25°C and the unidentified bacterial isolates were cultured on nutrient broth (NB) for 3 days. Then, the fungal and bacterial biomasses obtained from broth were centrifuged at 10000 rpm for 20 min, and the culture medium debris was discarded. Next, the supernatant was syringe filter sterilized (0.2 µm) and used for *in vitro* studies.

In vitro evaluation of antagonistic activities of culture filtrate of microflora by using slide spore germination test

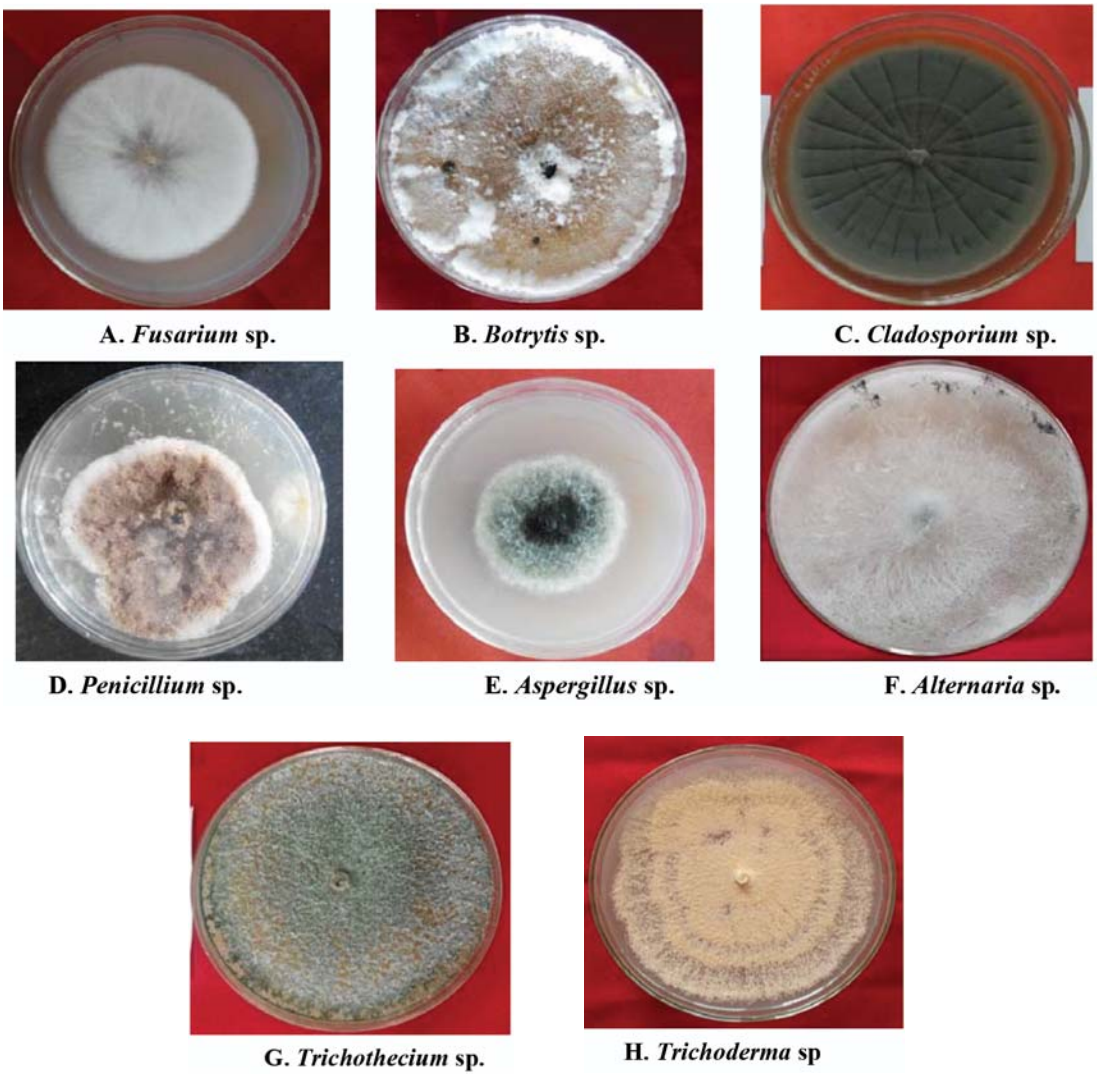


Fig. 1: (A-H). Fungal microflora isolated from rose phylloplane

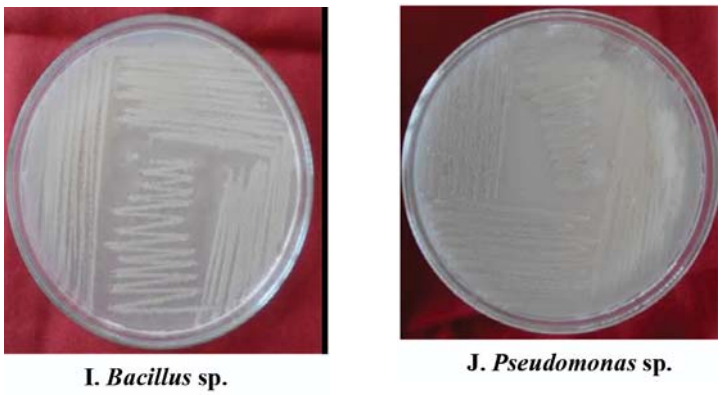
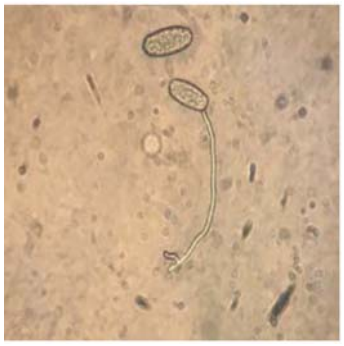
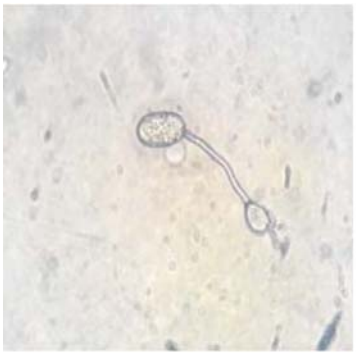


Fig. 2: (I-J). Fungal microflora isolated from rose phylloplane

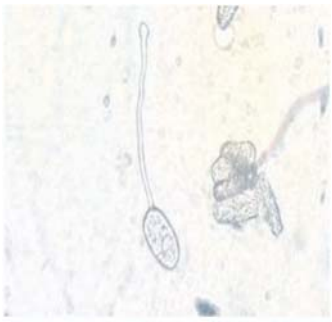
A. Showing emergence of germ tube



B. Production of secondary hyphae



C. Initial stage of an appresorium formation



D. Formation of an appresorium

Fig. 3: (A-D). Germ tube and appresorium production of conidia of *Podosphaera pannosa* under *in vitro* condition

Double strength solution of each culture filtrate was prepared in the sterilized distilled water. Simultaneously, the spore suspension of powdery mildew fungus (28-30 per microscopic field) was also prepared by dislodging the conidia from the infected leaves. One drop of each (i.e. culture filtrate and spore suspension) was separately put in the cavity of cavity slide, cavity slides containing mix were later placed on a glass rod, kept in a Petri plate containing sterilized distilled water at the bottom and sterilized moistened cotton wool lining the inner surface of the upper lid. Petri plates were then kept in the growth chamber for incubation at 25+1°C with 85 per cent relative humidity. The experiment was laid out in a Completely Randomized Design (CRD) and each treatment was replicated three times. The readings on germination of conidia were recorded after 24, 36, 48, 60 and 72 hrs by placing cavity slides under light microscope and per cent inhibition in germination of each culture filtrate was calculated by adopting the formula as given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition, C = Germination of conidia in control, T = Germination of conidia in treatment.

RESULTS AND DISCUSSION

Frequency of occurrence of rose phylloplane microflora

Present experiment was carried out to know the frequency of occurrence of the mycoparasites associated with rose powdery mildew and to evaluate their antagonistic properties against *Podosphaera pannosa*. Different microorganisms were isolated from phylloplane of the rose leaves which were *Fusarium* sp., *Botrytis* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Trichothecium* sp., *Trichoderma* sp. and two bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.) (Fig. 1 and 2). Frequency of occurrence of microorganisms in summer and winter seasons were observed during April, May, June, October, November and December months. Data pertaining to the frequency of occurrence of microorganisms given in table 1 showed that these fungal species *Fusarium*, *Aspergillus* and *Penicillium* and bacterial *Bacillus* sp. and *Pseudomonas* sp. had highest frequency of occurrence and isolated in all months of both summer and winter seasons. Lowest frequency was recorded in *Cladosporium* sp. which was isolated only in month of June followed by *Trichoderma* sp., isolated twice in May

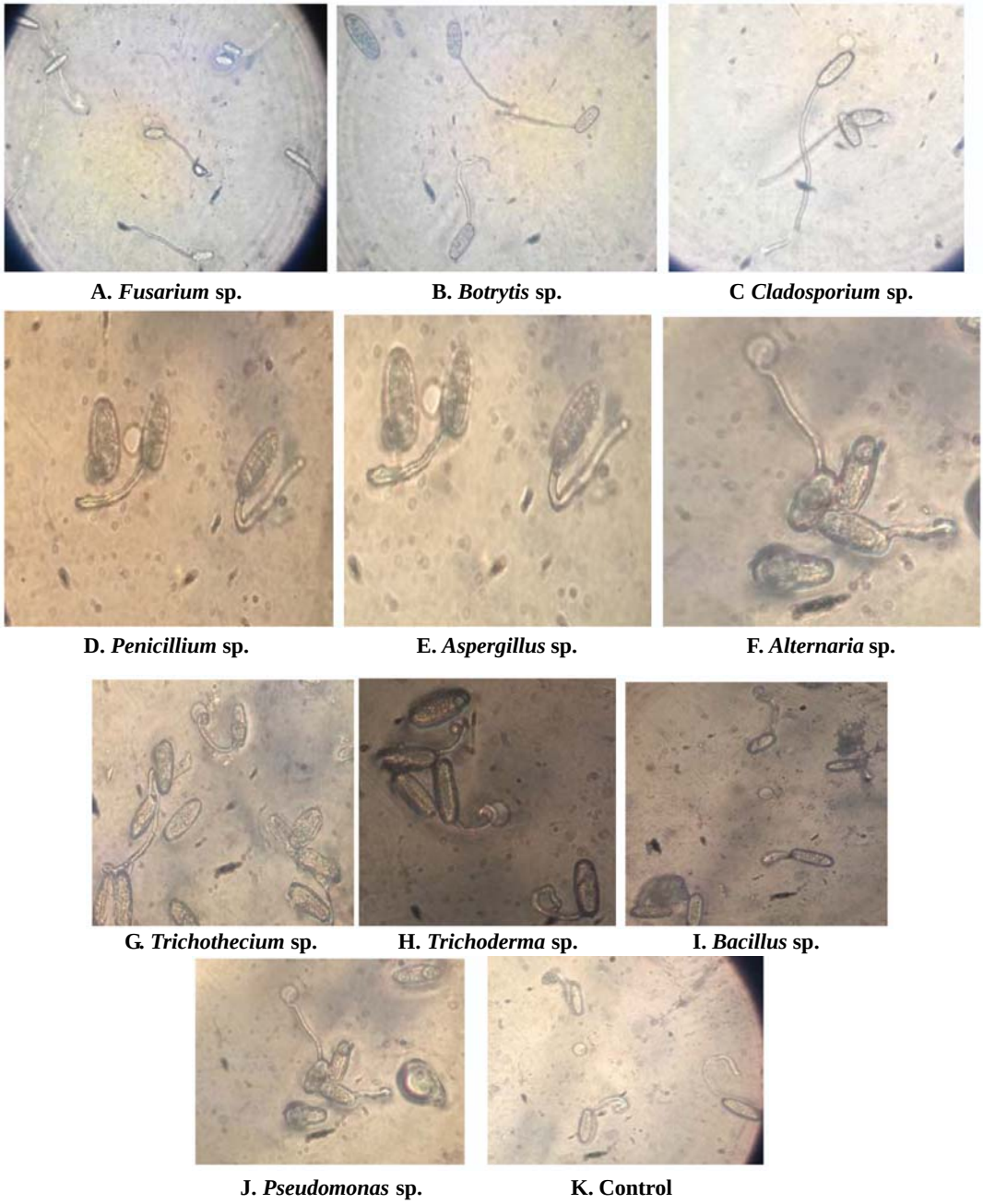


Fig. 4: (A-K). *In vitro* evaluation of phylloplane microflora culture filtrate through slide spore germination test against rose powdery mildew

and June months. Similar, observations were obtained by Yeasmin and Shamsi (2013) who isolated *Alternaria citrii, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Cladosporium cladosporoides, Colletotrichum capsici, Curvularia clavata, C. lunata, Fusarium nivale, F. semitectum, Penicillium* sp., *Pestalotia* sp., *Rhizopus stolonifer* and *Trichoderma viride* from the phylloplane of Gerbra. Tesfagiorgis and Laing (2010) had isolated 2,000 isolates of bacteria, fungi and yeasts from the phylloplane of powdery mildew infected zucchini plant. *Acremonium* sp., *Trichoderma* sp., *Ampelomyces* sp., *Phoma* sp. and *Leptosphaerulina australis* were commonly associated antagonists, associated with oak powdery mildew reported by Topalidou and Shaw (2015).

In vitro evaluation of antagonistic activities of the isolated microflora against powdery mildew pathogen (*Podosphaera pannosa*)

The information obtained from the *in vitro* antagonistic activities experiments shows that all the isolated microflora culture filtrate reduces the conidial germination upto 21.88 per cent (Table 2). The maximum conidial germination inhibition (21.88%) of *Podosphaera pannosa* was recorded in culture filtrate of the *Trichoderma* sp. followed by 16.20 per cent of *Alternaria* sp., *Botrytis* sp. (11.58%) and *Bacillus* sp. (11.45%). However *Botrytis* sp. and *Bacillus* sp. were significantly same with each other in expressing their antagonistic activity. The non effective culture filtrates with minimum conidial inhibition (2.66%) were reported from *Aspergillus* sp. followed by *Pseudomonas* sp. 2 (5.08%), *Penicillium* sp. (5.44%), *Cladosporium* sp. (7.98%), *Fusarium* sp. (8.68%) and *Trichothecium* sp. (10.89%) (Fig. 3 and 4). The present results were in accordance to Mmbaga *et al.* (2008) and Suman (2008). They found similar antagonistic fungi association as in above study *Penicillium* spp., *Trichoderma* spp., *Alternaria alternata*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium acuminatum*, *F. semitectum* and *Penicillium rubrum* while working with powdery mildew of flowering dogwood and *Trigonella foenum-graecum*, respectively and found *Trichoderma* spp. and *Alternaria alternata* best under *in vitro* slide germination test. Tesfagiorgis and Laing (2010) reported the antagonistic activities of the *Clonostachys rosea*, *Trichothecium roseum* and three isolates of *Serratia marcescens* against *Podosphaera xanthii*.

Trichoderma sp. gave the maximum conidial germination inhibition against *Podosphaera pannosa* but the isolated *Trichoderma* sp. was not as effective as the commercial formulation of *Trichoderma* are available in the market.

REFERENCES

Crespel, L. and Mouchotte, J. 2003. Methods of cross breeding. In: Roberts A, Debener T, Gudin S (eds): *Encyclopedia of Rose Science.* Elsevier Science, Oxford, pp 30-33.

Gudin, S. 2000. Rose: genetics and breeding. *Pl. Breed. Rev.* **17** : 59-189.

Maji, M.D, Qadri, S.M.H. and Pal, S.C. 2003. Evaluation of mulberry phylloplane microorganisms for biological control of bacterial leaf spot of mulberry caused by *Xanthomonas campestris* pv. *mori*. *Ind. J. Agric. Res.* **37(4) :** 307-09.

Mmbagaa M, Tsauveb, R.J. and Mremaa, F. A. 2008. Identification of microorganisms for biological control of powdery mildew in *Cornus florida. Biol. Control* **44(1) :** 67-72.

Suman, B. 2008. Inhibitory Effect of Phylloplane Fungi on *Erysiphe polygoni* DC inciting Powdery Mildew Disease of *Trigonella foenum-graecum. Ann. Pl. Prot. Sci.* **16(1) :** 150-52.

Tesfagiorgis, H.B. and Laing, M.D. 2010. Isolation and *in vitro* screening of potential biocontrol agents against powdery mildew of zucchini caused by *Podosphaera xanthii*. *S. Afr. J. Pl. Soil* **27(2):** 179-85.

Topalidou, E.T. and Shaw, M.W. 2015. Relationships between oak powdery mildew incidence and severity and commensal fungi. *Pathol.* **46(2):** 104-15.

Vincent, JH. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **150** : 850.

Yeasmin, F. and Shamsi, S. 2013. Phylloplane mycoflora of *Gerbera* spp. And their pathogenic potentiality. *J. Bangladesh Acad. Sci.* **37(2):** 211-17.

Zhang, L. 2003. Genetic linkage mapping in tetraploid and diploid rose. *Dissertation*, Clemson University, USA.