RESEARCH ARTICLE

Antifungal activity of gum-resin extracts of *Boswellia serrata*, *Commiphora mukul*, *Gardenia resinifera* and *Shorea robusta* against some plant pathogenic fungi

SMITA HENRY GAUREA^{1*}, SMRITY PRABHA² and UJWALA C. BAPAT¹

¹Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra, India

²Department of Botany, Ranchi College, Ranchi 834 008, Jharkhand, India

Received: 28 March 2017/ Accepted: 26 May 2017/ Published online: 31 May 2017

© Indian Phytopathological Society 2017

ABSTRACT: Resins are formed as oxidation products of various essential oils and are very complex and varied in chemical composition. They are usually secreted in definite cavities or passages. They frequently ooze out through the bark and harden on exposure to air. Resin production is widespread in nature and is reported from plants of Pinaceae, Araucariaceae, Umbelliferae, Dipterocarpaceae, Burseraceae, Rubiaceae and Sterculiaceae. Ethanolic extracts of gum resins of *Boswellia serrata* Roxb. ex Colebra, *Commiphora mukul* (Hooks ex Stocks) Engl., *Gardenia resinifera* Roth. and *Shorea robusta* Gaertn. were screened for their antifungal activity *in vitro* against phytopathogenic fungi, *Aspergillus niger* Tiegh., *Fusarium oxysporum* Schlecht., *Nigrospora oryzae* (Berk and Br.) Petch., *Rhizoctonia solani* J.G.Kuhn., and *Sclerotium rolfsii* Sacc and root rot fungus *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. The antifungal activity was tested by poisoned food technique. The results obtained showed that the growth of *R. solani* and *S. rolfsii* was stimulated by all the resin extracts. Extracts of *B. serrata* and *S. robusta* caused no inhibition of mycelial growth of the fungi tested. It was observed that the resin extract of *G. resinifera* was the most effective against *N. oryzae* and showed 77.16% inhibition of mycelia growth at the concentration of 111mg/ml.

Keywords: Antifungal activity, *Gardenia resinifera*, gum-resin, phytopathogenic fungi

Fungal phytopathogens pose serious problems worldwide and cause a number of plant diseases such as rusts, smuts, rots which ultimately lead to damage of crops. *Rhizopus stolonifer* is responsible for root rot in sugar beet (Hanson, 2010). *A. niger* is a saprophytic and filamentous fungus found in soil, organic debris and food products causing black mould of onion, garlic, stem rot of *Dracaena*, boll rot of cotton, spoilage of mango, cashew kernels, dates, figs, vanilla pods and crown rot of groundnut (Avasthi *et al.*, 2010; Bobbarala *et al.*, 2009; Gadgile and Chavan, 2011; Saifeldin *et al.*, 2016). Wilt caused by *F. oxysporum* is the most prevalent and damaging soil-borne disease of carnations in Himachal Pradesh (Raj *et al.*, 2016). *Fusarium* wilt of tomato and seedling damping-off caused by *F. oxysporum* cause serious economic losses to the producers (Al-Rahmah *et al.*, 2013; Neela *et al.*, 2014). The seed-borne disease of rice caused by *N. oryzae* shows symptom of grain spot and results in poor germination (Ahmed *et al.*, 2013). *Rhizoctonia solani* is a major pathogen, causing root rot disease in mothbeans (Patel *et al.*, 2016), sheath blight disease of rice (Lal *et al.*, 2012; Rout *et al.*, 2013; Tiwari and Das, 2011) and black scurf of potato (Singh and

Chaudhari, 2012) resulting in substantial yield losses. *S. rolfsii* is an omnivorous, soil borne pathogen causing sclerotial diseases in vegetables, legumes, cereals and stem and root rot of groundnut (Gour and Sharma, 2010; Kiran *et al.*, 2006; Vivek *et al.*, 2014).

Different measures have been employed for controlling phytopathogenic fungi (Das *et al.*, 2014). Various plant materials are believed to have antifungal activity (Riad and Aboelfetoh, 2014). In the recent years, interests have been generated in the development of safer anti-fungal agents from plant products to control fungal diseases.

Resins are amorphous mixtures of essential oils, oxygenated products of terpenes and carboxylic acids and are secreted as exudates from specialized structures in a wide range of plants. They probably function as plant defenses. There are very few references on the antifungal activity of resins against the plant pathogenic fungi. The present study was carried out to evaluate the antifungal activity of the resins of *B. serrata*, *C. mukul*, *G. resinifera*, *S. robusta* (since they are easily available and used in various ayurvedic preparations) against the plant pathogenic fungi such as *A. niger*, *F. oxysporum*, *N. oryzae*, *Rhizoctonia solani*, *S. rolfsii* and the rot fungus *Rhizopus stolonifer* (Table 1).

*Corresponding author: smitagaurea@gmail.com

Table 1. Fungal strains procured from Agharkar Research Institute, Pune

Fungal sp.	Strain	Source (isolated from)
<i>Rhizopus stolonifer</i>	NFCCI-1422	Marine soil
<i>Aspergillus niger</i>	NFCCI-3303	Coal mine soil
<i>Fusarium oxysporum</i>	NFCCI-2065	Decaying plant material
<i>Nigrospora oryzae</i>	NFCCI-2710	<i>Brassica</i> sp. root
<i>Rhizoctonia solani</i>	NFCCI-2662	Rice
<i>Sclerotium rolfsii</i>	NFCCI-2465	Unidentified nursery plant

MATERIAL AND METHODS

Plant materials

The resin of *Gardenia* was collected from Sanjay Gandhi National Park, Borivali (Maharashtra). The resins of *Boswellia* and *Commiphora* were collected from Jamboghoda (Gujarat) and that of *Shorea* from Panchmadi, (Madhya Pradesh). The morphological characters of plants from which these resins were collected were studied and the plants were identified by comparing with the Blatter Herbarium specimens and the description in the Flora of Maharashtra, Almeida (1996-2001) and Wealth of India (1948-1976). Herbarium sheets along with the resins were prepared for ready reference.

Preparation of resin extracts

Twenty percent extracts of resins were prepared by soaking overnight 10 g of each resin separately in 50 ml of Ethanol. The extracts were then filtered through Whatman No. 1 filter paper and then evaporated to dryness. The residues thus collected were dissolved separately in 10 ml of Ethanol and stored separately in glass bottles and used as samples. 0.2 ml of this sample was mixed with molten PDA followed by 0.8 ml of distilled water to obtain 20% extract. The extracts and the samples were prepared in triplicates.

Antifungal activity

The antifungal activity of resins was determined by food poison technique followed by Gupta *et al.* (2015). 0.05, 0.1, 0.15 and 0.2 ml of resin samples prepared in ethanol (as mentioned above) were diluted with 0.95, 0.9, 0.85 and 0.8 ml of water respectively. The diluted samples were added separately to molten PDA plates to get the final concentrations of 5, 10, 15 and 20%. PDA medium with 1 ml of 20% ethanol and 19ml of PDA medium served as control (-ve). Simultaneously 0.02ml of the antibiotic (streptomycin) solution was added to each assay plate to check the bacterial contamination. For each concentration, three replicates (plates) were kept. Bavistin (+ve control) was the standard fungicide used in the range of 5- 100 µg/ml. (as the inhibitory concentration of Bavistin for different fungi varies). A disc of 5mm of 7 days old culture of the fungus to be tested

was taken with a pre-sterilized cork borer and placed upside down at the centre of the medium in the dish with the solidified culture medium. The Petri plates were incubated at 25+2°C for 7 days. The radial growth of the colony was recorded on 2nd day for *Rhizopus stolonifer* (Zohra *et al.*, 2012) whereas for the rest of the fungi on 3rd, 5th and 7th day (Dwivedi *et al.*, 2012) and the % inhibition of mycelial growth was calculated by using following formula (Bapat *et al.*, 2016).

$$\% \text{ inhibition mycelial growth} = [C-T/C] \times 100$$

Where C and T are the growth diameter in (mm) in control (-ve) and treatment respectively.

Statistical Analysis

Two-way ANOVA analysis was carried out to compare the results obtained.

RESULTS AND DISCUSSION

The antifungal activity of resin samples prepared in ethanol was studied by measuring the colony diameter at 2/3, 5 and day 7. It showed following results.

There is an increase in the diameter of the fungal mycelial colony with the progression of days in all the cases including solvent (-ve) control and the resin extracts. Fig.1 indicates that *A. niger*, *F. oxysporum* and *N. oryzae* were sensitive to Bavistin at 100 µg/ml. Whereas, *Rhizopus stolonifer* and *Rhizoctonia solani* and *S. rolfsii* were resistant to Bavistin.

There was 62% inhibition of *Rhizopus stolonifer* at 100µg/ml of Bavistin. It was observed that *A. niger* and *F. oxysporum* were sensitive (100% inhibition) to Bavistin at 100 µg/ml. *N. oryzae* was very sensitive to Bavistin i.e. 100% inhibition of growth was observed at 10 µg/ml.

The extract of *B. serrata* did not inhibit the growth of any of the fungal plant pathogens tested. A similar result was observed by Mohan *et al.* (2011) with methanolic extract of *B. serrata* against *A. niger*. However, Sharma *et al.* (2016) observed the good antifungal activity of methanolic and ethyl acetate extracts of leaves, bark and fruits of *B. serrata* against *A. niger*.

The resin sample of *C. mukul* was not effective against *R. stolonifer*, *Rhizoctonia solani* and *S. rolfsii*. However, 60% inhibition of growth of *N. oryzae* and 25% inhibition of *A. niger* was observed at day 7. *F. oxysporum* was found to be very less sensitive (7% inhibition) to resin extract of *C. mukul*. There is no literature on the effect of resin extract on the growth of these fungi. However, Singh *et al.*, 2013 observed inhibition of *A. niger* at high concentration of alcoholic extracts of root, shoot and seeds of *C. wightii*. Whereas, Qureshi *et al.* (2015) reported that the leaf extract of *C. wightii* did not inhibit the growth of *A. niger*.

The resin samples of *G. resinifera* slowed down the growth of *R. stolonifer* initially, however later (from day 5), it showed full growth. The growth of *A. niger*, *F.*

Rhizopus stolonifer

Bavistin 100µg/ml

20% Ethanol

B. serrata *C. mukul* *G. resinifera* *S. robusta* ***A. niger***

Bavistin 100µg/ml

20% Ethanol

B. serrata *C. mukul* *G. resinifera* *S. robusta* ***F. oxysporum***

Bavistin 100µg/ml

20% Ethanol

B. serrata *C. mukul* *G. resinifera* *S. robusta* ***N. oryzae***

Bavistin 10µg/ml

20% Ethanol

B. serrata *C. mukul* *G. resinifera* *S. robusta*

Fig. 1. Antifungal activity of plant resin samples prepared in 20% ethanol against *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium oxysporum* and *Nigrospora oryzae* on PDA at 30 + 2°C at day 7

Fig. 2. Percent inhibition of six fungi at 7th day by gum resin samples prepared in ethanol

oxysporum and *N. oryzae* was inhibited by the resin sample of *G. resinifera*. Among these *N. oryzae* was found to be more sensitive (77% inhibition) to the extract of *G. resinifera* than the rest.

The resin sample (extract) of *S. robusta* showed results similar to the extract of *B. serrata* i.e. it did not inhibit the growth of the fungi tested. Similar results were obtained by Murthy *et al.* (2011) against *A. niger*. In contrast to this, Thampi *et al.* (2015) observed inhibition

of *A. niger* (18mm ZOI) by the chloroform extract of the same.

Among the four gum resins tested for their antifungal activity *in-vitro* against six plant pathogenic fungi, the resin samples of *G. resinifera* and *C. mukul* prepared in ethanol were effective against *N. oryzae* (Fig. 2). The % inhibition of *N. oryzae* by the resin sample of *G. resinifera* was the highest (77%) at day 7 (Fig. 2).

Statistical Analysis

Two-way ANOVA results suggested that among all the plants tested the resin sample of *G. resinifera* and *C. mukul* showed statistically significant inhibition at 0.01 level (1%). Between these two extracts, the % inhibition by *G. resinifera* was the most significant.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to The Principal, St. Xavier's College for providing the facilities. Authors are also thankful to Agharkar Research Institute, Pune for the supply of fungal culture. Thanks to Dr. Santhosh and Dr. Rashmi for their help in collection of the gum resins.

REFERENCES

- Ahmed M, Hossain M, Hassan K and Dash CK (2013). Efficacy of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. *Universal J. Plant Sci.* 1: 66-73.
- Al-Rahmah AN, Mostafa AA, Abdel-Megeed A, Yakout SM, Hussein SA (2013). Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *Afr. J. Microbiol. Res.* 7: 517-524.
- Almeida MR (1996-2001). Flora of Maharashtra, Volume I, p. 92, 218, 219, 146; Volume III A, p. 16. Mumbai: Orient Press.
- Avasthi S, Gautam AK and Bhadauria R (2010). Antifungal activity of plant products against *Aspergillus niger*: A potential application in control of a spoilage fungus. *Biological Forum - An Int. J.* 2: 53-55.
- Bapat UC, Smrity P and Jyoti K (2016). Antifungal activity of ethanolic and petroleum ether extracts of some medicinal plants against the plant pathogenic fungus *Sclerotium rolfsii* Sacc. *Int. J. Bioassays.* 5: 4714-4719.
- Bobbarala V, Katikala PK, Naidu KC and Penumajji S (2009). Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. *Indian J. Sci. Tec.* 2: 87-90.
- Das N, Ghosh A and Chatterjee P (2014). Antifungal effect of *Clitoria ternatea* L. leaf extracts on seeds of *Pisum sativum* L. in relation to some biochemical parameters. *Int. J. Res. Ayurv. Pharm.* 5: 536-539.
- Dwivedi SK and Dwivedi N (2012). Antifungal activity of some plant extracts against guava wilt pathogen. *Int. J. Environ. Sci.* 3: 412-420.
- Gadgil DP and Chavan AM (2011). Changes in Phosphorous content of mango pulp due to colonization of *Aspergillus niger*. *Indian Phytopath.* 64: 192-193.
- Gour HN and Sharma, P (2010). Evaluation of fungicides *in vitro* and *in vivo* against *Sclerotium rolfsii* Sacc. causing root rot of groundnut. *Indian Phytopath.* 63: 352-353.
- Gupta AK, Chaudhary S, Samuel CO and Upadhyaya PP (2015). Antifungal activity of some locally available plants against *Fusarium oxysporum* f. sp. Udm. *Int. J. Curr. Microbiol. App. Sci.* 4: 695-699.
- Hanson LB (2010). Interaction of *Rhizoctonia solani* and *Rhizopus stolonifer* causing root rot of sugar beet. *American Phytopath. Soc.* 94: 504-509.
- Kiran KS, Lingaraju S and Adiver SS (2006). Effect of plant extracts on *Sclerotium rolfsii*, the incitant of stem rot of groundnut. *J. Mycol. Pl. Pathol.* 36: 77-79.
- Lal M, Kandhari J and Singh V (2012). Characterization of virulence pattern in *Rhizoctonia solani* causing sheath blight of rice. *Indian Phytopath.* 65: 60-63.
- Murthy KSR, Lakshmi N and Ramulu DR (2011). Biological activity and phytochemical screening of the oleoresin of *Shorea robusta* Gaertn. f. *Tropical and Subtropical Agroecosystems* 14: 787-791.
- Neela FA, Sonia IA and Shamsi S (2014). Antifungal activity of selected medicinal plant extract on *Fusarium oxysporum* Schlecht the causal agent of *Fusarium* wilt disease in tomato. *American J. Plant Sci.* 5: 2665-2671.
- Patel DB, Singh N, and Chaudhari SM (2016). Efficacy of seed dressing agents against root rot (*Rhizoctonia solani*) of mothbean (*Vigna aconitifolia*). *Indian Phytopath.* 69: 137-140.
- Qureshi I and Chahar OP (2015). Antimicrobial activity of guggul (*C. wightii*) plant in arid region of Jhunjunu. *Int. J. Engineering Res. Management Tech.* 2: 37-41.
- Raj H, Chauhan P and Chandel S (2016). Integrated management of carnation wilt caused by *Fusarium oxysporum* f. sp. Dianthi using soil solarisation and soil amendments. *Indian Phytopath.* 69: 396-399.
- Riad SRE and Aboelfetoh MA (2014). Antifungal activity of *Moringa oleefera* oil and seed extract against some plant pathogenic fungi Middle East. *J. Agric. Res.* 3: 242-249.
- Rout S, Thatoi HN and Tewari SN (2013). Combating sheath blight pathogen (*Rhizoctonia solani*) of rice with *Aegle marmelos* extract based Amalab, a formulated botanical product. *Indian Phytopath.* 66: 168-171.
- Saifeldin AFE, Awad HMA and Abdulkadi EE (2016). In vitro evaluation of selected plant extracts as biocontrol agents against black mold (*Aspergillus niger* Van Tieghem) of onion bulbs (*Allium cepa* L.). *Int. J. Sci. Tech. Res.* 5: 147-152.
- Sharma M, Jain A and Barolia SK (2016). In vitro antimicrobial screening of *Boswellia serrata*. *Int. J. Advanced Res.* 4: 1594-1602.
- Singh N and Chaudhari SM (2012). Management of black scurf (*Rhizoctonia solani*) and common scab (*Streptomyces scabies*) of potato through eco-friendly components. *Indian Phytopath.* 65: 378-381.
- Thampi N and Kumar N (2015). Evaluation of antimicrobial and free radical scavenging potentials of three valuable medicinal plants- *Hyptis suaveolens*, *Spathodea campanulata* and *Shorea robusta*. *J. Chem. Pharma. Res.* 7: 609-615.
- Tiwari RKS and Das K (2011). Inhibitory effect of cow urine based plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *Indian Phytopath.* 64: 265-268.
- Vivek MN, Manasa M, Yoshada K, Noor NAS, Vinayaka KS and Prashith KTR (2014). Antifungal activity of some plants of Western Ghats of Karnataka against *Sclerotium rolfsii*. *Indian J. Advances Plant Res.* 1: 29-33.
- Wealth of India (1948-1976). A dictionary of Indian Raw materials and Industrial products, Volume I, p. 208- 210; Volume II, p. 313-314; Volume IV, p. 111; vol IX, p. 312-322; Volume X, p. 43-48. New Delhi: Council of Scientific and Industrial Research, Publication and information directorate.
- Zohra SF, Meriem B, Chokri B and Alsayadi MMS (2012). Evaluation of antifungal activity of some extracts from Mallow. *J. Microbio. Biotech. Res.* 2: 603-605.