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In vitro Antifungal Activity of Some Plants Against *Bipolaris sorokiniana* (Sacc.) Shoem

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A B S T R A C T

Bipolaris sorokiniana (Sacc.) Shoem. is a serious pathogen of wheat and causes root rot disease. The present study was carried out to determine inhibitory activity of 40 extracts from 37 plants belonging to 34 genera and 19 families against *B. sorokiniana* by Poisoned food technique. All extracts exhibited marked antifungal activity in terms of inhibition of mycelial growth of fungus. The percentage inhibition of fungus caused by extracts ranged between 54 to 91%. Extract of 8 plants *viz.*, *Curcuma aromatica*, *Atylosia lineata*, *Butea superba*, *Striga gesnerioides*, *Calceolaria mexicana*, *Adhatoda vasica*, *Conyza stricta* and *Smithia sensitiva* exhibited highest inhibitory activity (>90% inhibition). Least antifungal effect was displayed by extract of *Emilia sanchifolia* (54% inhibition). These extracts appear promising antifungal agents. Further investigations in field conditions are to be carried out.

Introduction

Fungi are important in agriculture as they are involved in improvement of crop production as well as destruction of crops. Among plant pathogens, fungi are the important pathogens as they cause huge number of diseases in crops leading to economic loss. Various approaches are used for controlling phytopathogenic fungi. The use of synthetic fungicides is the commonly used strategy. However, the use of these conventional synthetic chemicals resulted in environmental pollution, soil and ground water toxicity, development of resistant strains of the fungal pathogens, slow biodegradation and effect on non-target organisms including humans. On entry into humans, these chemicals cause severe health effects. Control of phytopathogens using botanicals is one of the best alternatives for chemical fungicides. They are cost effective,

safer and ecofriendly. Higher plants are shown to possess fungitoxic activity against a range of pathogenic fungi (Seema *et al*., 2011; Boonsang *et al*., 2014; Kambar *et al*., 2014; Rafi and Dawar, 2015; Shweta *et al*., 2015; Parveen *et al*., 2016).

Wheat is one among the staple cereal food crops consumed in most parts of the world. The production of crop is threatened by several disease causing agents. *Bipolaris sorokiniana* (Sacc.) Shoem is a serious pathogen of wheat. It causes disease symptoms such as root rot, leaf blight, seedling blight and spot blotch. The pathogen affects seed germination and seedling emergence and causes considerable reduction in grain yield. The disease is a serious threat for wheat cultivation especially in wet subtropical climatic regions. High temperature and high humidity favors for the disease outbreak. The initial symptoms of root rot originate on young seedlings from inoculum carried on the seed or from soilborne conidia near the seedling. Dark brown lesions are formed on outer coleoptile tissue and/or on the leaf base. Lesions may unite into long areas of necrotic brown tissue. The entire seedling may die in severe infection. In most cases, the seedling will survive but plant growth is stunted.

The management of disease is usually achieved by using chemicals. Plant extracts and plant based formulations appear promising in the control of phytopathogenic fungi including *B. sorokiniana* (Mathre *et al*., 2003; Salehpour *et al*., 2005; Akhter *et al*., 2006; Acharya *et al*., 2011; Hasan *et al*., 2012; Perelló *et al*., 2013a; Hossain *et al*., 2015; Bahadar *et al*., 2016). In the present study, we determined inhibitory effect of extracts from 37 plants collected from different places of Karnataka, India.

Materials and Methods

Collection and Identification of Plants

A total of 37 plants belonging to 34 genera and 19 families used in this study were collected from different places of Karnataka *viz*., Lakkavalli, Shankaraghatta, Mullayanagiri, Bababudangiri, Sagara, Chikkamagalure, Varadahalli, Haniya and Thirthahalli. Identification of plants was made by referring standard flora (Saldhana and Nicolson, 1978; Yoganarasimhan *et al*., 1981; Bhat, 2014) and with the help of taxonomists. Table 1 presents details of the plants *viz*., name, family and parts of the plants used.

Extraction

The collected plants were washed using clean water to remove adhering matter. The plant materials were dried under shade to prevent loss of volatile constituents and powdered in a blender. Extraction of powdered plant materials was done by simple maceration process where a known quantity of material (10g) was transferred into clean conical flask containing 100ml of methanol. The flasks were sealed and left for 48 hours with stirrings done occasionally. The contents of flasks were filtered through Whatman No. 1 filter paper and the filtrates were evaporated to dryness in oven at 50^{o}C (Kekuda *et al*., 2015).

Antifungal Activity of Extracts

Suppressive effect of extracts of selected plants on radial mycelial growth of *B. sorokiniana* was determined by Poisoned food technique. Control (without extract) and poisoned (2mg extract/ml of medium) Potato dextrose agar plates were aseptically inoculated with well sporulated culture of fungus by point inoculation method. The

plates were incubated at 28°C for 5 days. The diameter of radial growth of fungus on plates was measured using a ruler in mutual perpendicular directions. Antifungal effect of extracts in terms of suppression of mycelial growth of fungus was determined using the formula:

Inhibition of radial growth (%) = (C – T / C) x 100, where C and T denotes the diameter of fungal colonies on control and poisoned plates respectively (Kekuda *et al*., 2015).

Results and Discussion

The crop cultivation and the development of human civilization have been closely linked. Diseases of crops became a serious concern to mankind ever since plants have been cultivated for various needs, in particular for consumption. At the end of the 19th and the beginning of the 20th century, chemical protection measures were developed which were proven to be so effective in reducing disease incidence. However, a few years later, emergence of resistant strains of fungal pathogens have been observed. Fungi exhibit resistance to chemicals by mutations or by various mechanisms that are induced by sublethal fungicide stress (Deising *et al*., 2008; Hahn, 2014). Moreover, the usage of fungicides of chemical origin resulted in environmental pollution and toxic effects on non-target organisms including humans. High cost, possible adverse health effects and environmental pollution aspects of these fungicides triggered immense interest for searching alternatives for chemical fungicides. Botanicals are shown to be one of the effective alternatives for chemical agents. Researches done on several plant species have shown marked suppressive effect of plants against several phytopathogenic fungi (Seema *et al*., 2011; Kambar *et al*., 2014; Vivek *et al*., 2014; Shweta *et al*., 2015; Parveen *et al*., 2016). In the present study, we evaluated the antifungal effect of 40 extracts from 37 plants against the fungus *B. sorokiniana*. Table 2 and Figure 1 depict the inhibitory activity (%) of extracts against mycelial growth of the test fungus. All extracts were effective in inhibiting the mycelial growth of the fungus but to a varied extent. An inhibition of >50% was recorded in case of all extracts. The percentage inhibition of test fungus by extracts was in the range 54.16% to 91.66%. An inhibition of 60 to 70% was exhibited by extract of *E. mysoriensis*. Extract from six plants displayed an inhibitory activity in the range 70 to 80%. 24 out of 40 extracts showed inhibitory activity in the range 80 to 90%. Extract from 8 plants *viz*., *C. aromatica*, *A. lineata*, *B. superba*, *S. gesnerioides*, *C. Mexicana*, *A. vasica* (leaf), *C. stricta* and *S. sensitiva* exhibited highest activity i.e., 91.66% inhibition of the fungus. Least inhibitory activity was observed in case of *E. sanchifolia* (54.16%). Similar studies on inhibitory activity of plants against *B. sorokiniana* have been carried out by some researchers. Extracts of *Vinca rosea* and *Azadirachta indica* exhibited inhibitory activity against germination of spores of *B. sorokiniana* (Alam *et al*., 2002). A study by Akhter *et al*. (2006) showed the efficacy of some plant extracts to inhibit germination of spores of *B. sorokiniana*. Extract of *Adhatoda vasica* and *Zingiber officinale* were shown to cause 100% inhibition of spore germination. In our study, leaf and flower extract of *A. vasica* exhibited marked inhibition of mycelial growth of *B. sorokiniana*. The studies carried out by Perelló *et al*. (2013a and 2013b) showed the efficacy of allicin and garlic juice containing allicin to inhibit *B. sorokiniana*.

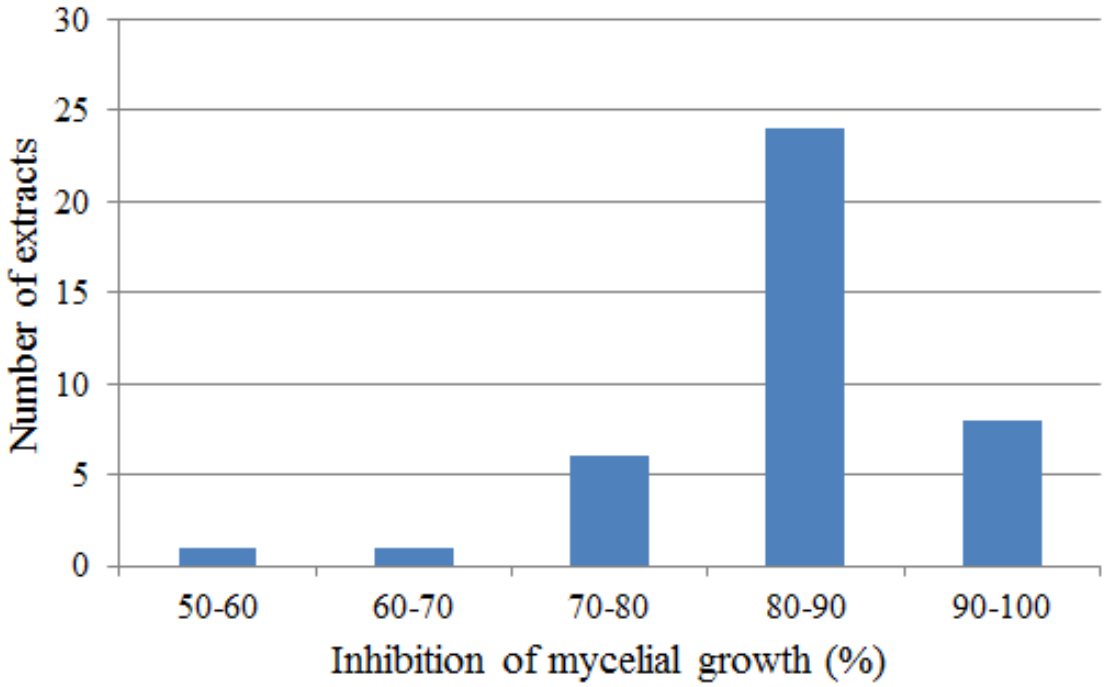
Table.1 Plants selected in this study

Sl. No.	Name	Family	Part used	Sl. No.	Name	Family	Part used
1	*Strobilanthes kunthiana* T.Anderson ex Benth.	Acanthaceae	Leaf	20	*Emilia sanchifolia* (L.) DC. ex DC.	Compositae	Whole plant
2	*Luisia macrantha* Blatt. McCann.	Orchidaceae	Whole plant	21	*Striga gesnerioides* (Willd.) Vatke	Orobanchaceae	Whole plant
3	*Dendrobium herbaceum* Lindl.	Orchidaceae	Whole plant	22	*Calceolaria Mexicana* Benth.	Calceolariaceae	Whole plant
4	*Oberonia brunoniana* Wight	Orchidaceae	Whole plant	23	*Strobilanthes sessilis* Nees	Acanthaceae	Leaf
5	*Bulbophyllum neilgherrense* Wight	Orchidaceae	Whole plant	24	*Argyreia speciosa* (L. f.) Sweet	Convolvulaceae	Leaf and flower
6	*Bulbophyllum fischeri* Seidenf.	Orchidaceae	Whole plant	25	*Adhatoda vasica* Nees	Acanthaceae	Leaf and flower
7	*Eria mysorensis* Lindl.	Orchidaceae	Whole plant	26	*Syzygium leatum* (Buch.-Ham.) Gandhi	Myrtaceae	Leaf
8	*Acampe praemorsa* (Roxb.) Blatter & McCann	Orchidaceae	Whole plant	27	*Swertia lawii* Burkill	Gentianaceae	Whole plant
9	*Coelogyne nervosa* A.Rich.	Orchidaceae	Whole plant	28	*Conyza stricta* Willd.	Compositae	Whole plant
10	*Vanda roxburghii* R.Br.	Orchidaceae	Whole plant	29	*Hoya wightii* Hook.f.	Apocyanaceae	Leaf
11	*Pholidota imbricata* Lindl.	Orchidaceae	Whole plant	30	*Hoya ovalifolia* Wight & Arn.	Apocyanaceae	Leaf
12	*Nardostachys jatamansi* (D. Don) DC.	Caprifoliaceae	Rhizome	31	*Leucas marruboides* Desf.	Labiatae	Root
13	*Curcuma aromatica* Salisb.	Zingiberaceae	Rhizome	32	*Smithia sensitiva* Aiton	Leguminosae	Whole plant
14	*Putranjiva roxburghii* Wall	Putranjivaceae	Seed	33	*Crotalaria filipes* Benth.	Leguminosae	Whole plant
15	*Fahrenheitia zeylanica* (Thwaites) Airy Shaw	Euphorbiaceae	Leaf	34	*Polyalthia longifolia* (Sonn.) Thwaites	Annonaceae	Ripe and unripe pericarp
16	*Helichrysum buddledoides* DC.	Compositae	Whole plant	35	*Maesa indica* (Roxb.) A. DC.	Primulaceae	Leaf
17	*Atylosia lineata* Wt. & Arn.	Leguminosae	Leaf	36	*Anaphalis lawii* (Hook.f.) Gamble	Compositae	Whole plant
18	*Butea superba* Roxb.	Leguminosae	Leaf	37	*Hypericum mysorense* B.Heyne ex Wight & Arn.	Hypericaceae	Leaf
19	*Coscinium fenestratum* (Goetgh.) Colebr.	Menispermaceae	Stem				

Table.2 Inhibitory activity of extract of selected plants against *B. sorokiniana*

Sl. No.	Treatment	Inhibition (%)	Sl. No.	Treatment	Inhibition (%)
1	*S. kunthiana*	87.50	21	*S. gesnerioides*	91.66
2	*L. macrantha*	87.50	22	*C. Mexicana*	91.66
3	*D. herbaceum*	87.50	23	*S. sessilis*	83.33
4	*O. brunoniana*	79.16	24	*A. speciosa* leaf	87.50
5	*B. neilgherrense*	70.33	25	*A. speciosa* flower	83.33
6	*B. fischeri*	79.16	26	*A. vasica* leaf	91.66
7	*E. mysoriensis*	62.50	27	*A. vasica* flower	87.50
8	*A. praemorsa*	75.00	28	*S. leatum*	83.33
9	*C. nervosa*	77.08	29	*S. lawii*	87.50
10	*V. roxburghii*	85.41	30	*C. stricta*	91.66
11	*P. imbricate*	79.16	31	*H. wightii*	83.33
12	*N. jatamansi*	89.58	32	*H. ovalifolia*	87.50
13	*C. aromatica*	91.66	33	*L. marruboides*	89.58
14	*P. roxburghii*	87.50	34	*S. sensitiva*	91.66
15	*F. zeylanica*	87.50	35	*C. filipes*	87.50
16	*H. buddledoides*	87.50	36	*P. longifolia* ripe pericarp	85.41
17	*A. lineata*	91.66	37	*P. longifolia* unripe pericarp	83.33
18	*B. superba*	91.66	38	*M. indica*	83.33
19	*C. fenestratum*	89.58	39	*H. mysoriensis*	87.50
20	*E. sanchifolia*	54.16	40	*A. lawii*	81.25

Fig.1 Range of mycelial inhibition of *B. sorokiniana* by extracts



Study of Hasan *et al*. (2012) revealed dose dependent inhibition of *B. sorokiniana* by extract of 5 plants. Hossain *et al*. (2015) showed antifungal efficacy of 13 plants against *B. sorokiniana*. More recently, essential oil of flowering buds and extracts from leaf, bark and flowering buds of *Eucalyptus camaldulensis* were shown to exhibit potent inhibitory activity against mycelial growth and spore germination of *B. sorokiniana* (Bahadar *et al*., 2016).

In conclusion, botanical extracts are known to exhibit potent inhibitory activity against a wide range of phytopathogenic fungi and can be considered as a promising alternative for chemical fungicides. In the present study, all extracts have shown to exhibit marked antifungal activity against *B. sorokiniana*, causal agent of root rot of wheat. These plants can be used to control root rot disease of wheat. From the observations of the present study, it can be suggested that investigations on antifungal effect of these botanicals are to be carried out in field conditions to develop fungicidal formulations.

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