EC MICROBIOLOGY
Research Article

Antifungal Activity of *Helichrysum buddleioides* DC. against Seed Borne Fungi

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Received: February 11, 2017; **Published:** February 20, 2017

Abstract

Seeds are considered as important inputs for production of several valuable crops. Seeds often carry pathogenic fungi which cause deterioration of seeds, reduction in seed viability and disease in seedlings and other stages of growth. The present study was conducted to investigate the inhibitory effect of *Helichrysum buddleioides* DC. (*Asteraceae*) against fungi isolated from sorghum and paddy seeds. A total of 12 fungi were screened for their susceptibility to extract of *H. buddleioides* by Poisoned food technique. Extract inhibited all fungi dose dependently. Marked and least inhibitory effect was observed against *Alternaria alternata* and *Rhizopus* sp. and *Cladosporium* sp. respectively. At 2 mg/ml concentration of extract, the extent of inhibition of fungi observed was > 50%. The plant can be exploited in the management of seed borne fungi and for prevention of deterioration of seeds and mycotoxin production during storage.

Keywords: *Helichrysum buddleioides; Antifungal; Seed-Borne Fungi; Poisoned Food Technique*

Introduction

Seed is an important input for production of many valuable crops and majority (about 90%) of the food crops are propagated by seed. The seeds should be of good quality and free from pathogens. The use of such healthy seeds results in desired germination and emergence of plants. However seeds act as the passive carriers of several infectious fungi which cause diseases in seedlings and other stages of growth and result in considerable yield losses. Species of *Aspergillus, Fusarium, Helminthosporium, Curvularia, Alternaria, Epicoccum, Mucor, Rhizopus, Cercospora, Pyricularia, Rhizoctonia* etc. are frequently found seed borne fungi. These seed borne fungi that are found on the surface and inside the seeds are one of the important constraints resulting in the deterioration of seed quality. These fungi cause reduction in the nutritive value of seeds and reduce seed viability [1-8].

The use of synthetic agents (fungicides) for the control of phytopathogenic fungi has undoubtedly resulted in marked plant protection, however, their usage is associated with drawbacks such as resistance in fungi, environmental pollution and adverse effect on non-target organisms including humans. These chemicals are non-biodegradable and their reckless use results destruction of useful soil microflora and loss of biodiversity. Moreover, the grains stored for consumption are generally not treated with chemical agents due to possible toxicity on consumption [9-12]. There is an upsurge interest in finding alternatives for pathogen control. Biological control of phytopathogens is one of the widely researched areas that focus on strategies such as utilization of antagonistic organisms and plants and plant based formulations. Plant metabolites and plant based formulations are shown to be better alternatives for chemical fungicides in crop protection and prevention of mycotoxin synthesis and biodeterioration grains caused by phytopathogenic fungi [8,12-17].

Helichrysum buddleioides DC. belongs to the family *Asteraceae* and is an under shrub, up to 1 meter tall. The plant is distributed in Western Ghats, Mysore, Bababooduns, Anamalais and Ceylon. Leaves are broad, oblong-oblanceolate, thin, apex obtuse-acute, nerves not impressed. Heads form dense, terminal corymbs. Involucral bracts are yellow, 4-many-seriate, woolly tomentum. Ray florets are female, filiform and 2.5 mm long, 4-lobed. Disc florets are bisexual, corolla are 2 mm long and 5-lobed. Achenes are 0.3 mm long, puberulous, pappus of white hairs present. Flowering and fruiting occurs between July and March [18,19]. The plant juice is used by Badaga population in the Nilgiri district of Tamilnadu to treat cut and wounds for rapid healing [20]. In an earlier study by Kekuda., *et al*. [21] the whole plant extract was shown to exhibit antifungal activity against *Bipolaris sorokiniana*. The present study was conducted to evaluate antifungal potential of extract of *H. buddleioides* against mycoflora isolated from seeds of sorghum and paddy.

Materials and Methods

Collection and extraction of plant material

The plant was collected in and around Bababudan giri, Chikmagalur, Karnataka in the month of November 2015. Identification was carried out by referring standard flora [18,19]. The plant was shade dried, powdered and extracted by maceration process using methanol [21].

Test fungi

A total of 12 fungi namely *Alternaria alternata, Curvularia lunata, Helminthosporium* sp., *Cladosporium* sp., *Rhizopus* sp., *Mucor* sp., *Aspergillus niger, A. flavus, A. fumigatus, Drechslera* sp., F*usarium oxysporum, F. moniliforme* and *Penicillium* sp. were tested for their susceptibility to extract of *H. buddleioides*. These fungi were isolated from seeds of sorghum and paddy by standard blotter method and agar plate method.

The identification of fungi was made on the basis of cultural, mycelial and spore characteristics. The fungi were maintained on Potato dextrose agar (PDA) slants.

Antifungal activity of extract

To evaluate antifungal potential of extract of *H. buddleioides*, we performed Poisoned food technique. The extract was amended into PDA medium at different concentrations (0.1, 0.5, 1.0 and 2.0 mg extract/ml of PDA). The test fungi were inoculated aseptically (5 mm discs) on control (without extract) and poisoned PDA plates followed by incubating the plates at room temperature for 5 days. The diameter of fungal colonies on control and poisoned plates was measured in mutual perpendicular directions. Antifungal activity of extracts in terms of inhibition of mycelial growth of test fungi (%) was determined using the formula:

Inhibition of fungal growth (%) = (C – T / C) x 100, where C and T denotes the mycelial diameter of test fungi on control and poisoned plates respectively [21].

Results and Discussion

Fungi are ubiquitous and infection due to phytopathogenic fungi is more common in field as well as storage conditions [22]. Seed associated fungi cause seed abortion, seed rot, seed necrosis, reduction of nutritive value and germination capacity and seedling damage. Seed treatment is the safest and cheap method to prevent deterioration of seed quality and crop loss in fields. Studies have shown that seed treatment or dressing with fungicides and other agents such as plant extract have shown reduced incidence of seed borne fungi and promotion of germination and seedling growth [4,7-9,23,24]. Extracts and purified compounds from plants are promising in terms of their potential to inhibit several seed borne fungi [4,12,14,15,17,25-27]. In the present study, the antifungal potential of different concentrations of extract of *H. buddleioides* was screened against 12 fungi isolated from seeds by poisoned food technique. This technique is widely used to evaluate antifungal activity of plant extracts [8,14,25,28,29].

The result of antifungal effect of extract is shown in Table 1 and Figure 1. Incorporation of extract into the medium and growing of fungi in poisoned medium resulted in dose dependent suppression of mycelial growth of fungi. Reduction in mycelial diameter is an indication of antifungal activity of extract. The extract was effective against fungi. At 2 mg/ml concentration, all fungi were inhibited to > 50%. Out of 12, 8 fungi were inhibited to 50% and higher at 1 mg/ml concentration of extract. At 0.1 mg/ml concentration, *A. flavus* was unaffected. Among fungi, *A. alternata* was inhibited to higher extent by extract whereas *Rhizopus* sp. and *Cladosporium* sp. were inhibited to least extent. More recently, Kekuda., *et al*. [21] showed the antifungal potential of *H. buddleioides* against *Bipolaris sorokiniana*. Studies have revealed the inhibitory effect of botanicals against mycoflora from various kinds of seeds such as brinjal [23], sorghum [14], maize [14], paddy [14,30], soyabean [10], tomato [29], sesame [31], Faba bean [32], ground nut [33], suflower [34] and barley [8].

Test fungi	Colony diameter in cm at different concentrations of extract				
	0.0 mg/ml	0.1 mg/ml	0.5 mg/ml	1.0 mg/ml	2.0 mg/ml
A. alternata	3.8	3.2	1.8	1.2	0.7
C. lunata	4.0	3.8	3.2	2.0	0.9
Helminthosporium sp.	4.2	4.0	3.6	2.1	1.2
Cladosporium sp.	3.4	3.3	3.1	2.2	1.4
Rhizopus sp.	5.4	5.1	4.4	3.6	2.2
Mucor sp.	5.6	5.0	4.2	3.0	2.0
A. niger	4.2	4.0	3.2	2.0	1.0
A. flavus	3.9	3.9	3.1	2.1	1.1
A. fumigatus	3.6	3.1	2.5	1.5	0.8
F. oxysporum	4.8	4.4	3.2	2.3	1.2
F. moniliforme	4.4	4.0	3.1	2.1	1.1
Penicillium sp.	4.1	3.6	3.0	1.8	0.8

Table 1: *Antifungal activity of H. buddleioides extract.*

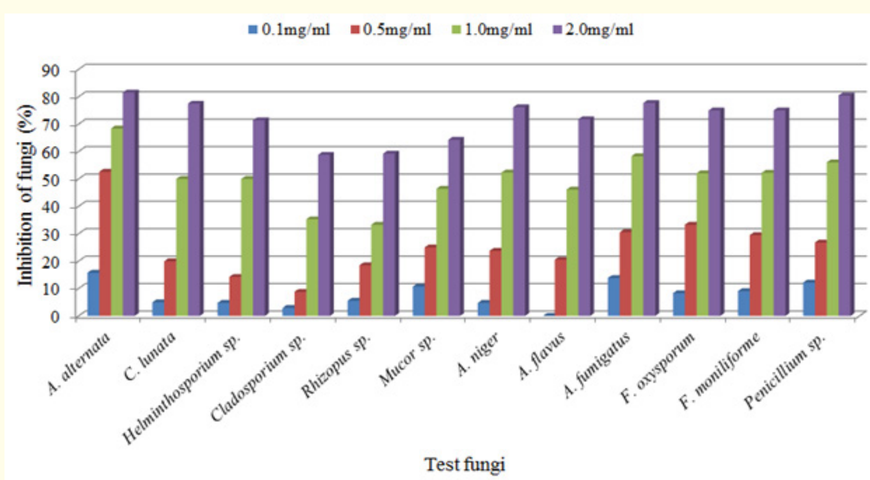

Figure 1: *Extent of inhibition of test fungi by different concentrations of extract.*

Conclusion

Seed treatment does not replace the availability and use of healthy seeds but it is considered as an effective way to increase seedling emergence especially when seed is of low vigor and associated with pathogens. Botanicals are known to be cost-effective, non-toxic, eco-friendly agents for control of phytopathogenic fungi. The extract of *H. buddleioides* showed marked inhibitory activity against a seed borne fungi and the inhibitory activity observed highlights the presence of antifungal principles in the extract. In suitable form, the plant can be exploited against seed borne fungi and for prevention of deterioration of seeds and mycotoxin production during storage.

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