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Anti-fungal activity of plant products against *Aspergillus parasiticus*: An exploration *in vitro*

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ABSTRACT

The present investigation was designed to evaluate the anti-fungal potential of commonly available plant products against *Aspergillus parasiticus*, an aflatoxin producing fungi. Plant products (63) were selected for the study. The test fungal species was grown in medium with and without the plant products and the growth was monitored up to 5 days of incubation. The results showed that maximum antifungal activity was demonstrated by oil of *Syzygium aromaticum* followed by *Cymbopogan citratus. Curcuma longa* and *Piper nigrum* produced tiny colonies without spores. The pulp of *Zingiber officinale, Capsicum frutescens,* leaves of *Azadirachta indica, Cyanodon dactylon, Citrus limonum, Ocimum sanctum,* seeds of *Terminalia chebula* and *Myristica fragrans* inhibited *A. parasiticus* growth by 7–20%. The study indicated the efficiency of specific plant products to inhibit the growth of the aflatoxin producing *Aspergillus* fungi. To fully exploit products for the benefit of animal performance, active ingredients and their modes of action need to be understood.

Key words: Anti-fungal activity, *Aspergillus parasiticus*, Plant products

Quality of feed is one of the main factors that determine the performance of animals, hence, any factor affecting feed quality draws attention. Infection by fungi, one of the major factors affecting the quality of feeds, results in reduction in yield and quality with significant economic losses. Certain fungi also produce secondary metabolites called mycotoxins on feeds (Huwig *et al.* 2001, Aksoy *et al.* 2009, Umaya *et al.* 2010); the most predominant and toxic is the aflatoxin, produced by *Aspergillus* fungi. The economic impact of feed contamination with aflatoxins includes organ damage, reduction in productivity and mortality (Upadhaya *et al.* 2010). Aflatoxin is very challenging mycotoxins as it could be produced by the responsible fungi prior to harvest and at post harvest stages including storage. More than 60% contamination was reported by aflatoxin (Diaz *et al.* 2009, Elzupir *et al.* 2009, Umaya *et al.* 2010). Aflatoxin is the single most important contaminant on the Rapid Alert System for Food and Feed (RASFF) of the European Union (Energy 2009). Some of the metabolites of aflatoxin in animals are carried over to milk, meat and egg (Bintvihok and Davitiyananda 2002, Fernandez *et al.* 2006).

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Indiscriminate use of fungicides has led to medical problems due to residual toxicity (Agboola *et al.* 2012) and they are not environment friendly too. Hence there is a need to search for alternative approaches to store grains/cereals for animal consumption. Newer methods or products that are not capital intensive, ecofriendly and without toxicity problems are desirable (Zahid *et al.* 2012). In India, plants are used for treatment of various diseases and the present consumers demand for animal products that are organic in nature. Various plant products have been reported to exhibit antimicrobial effects and more specifically, anti-fungal activities against *Aspergillus niger*, *A. candidus*, *A. columnaris*, *A. flavipes, A. flavus*, and *A. fumigatus* under *in vitro* conditions (Satish *et al.* 2007). Hence plant products appear as ideal candidates to control fungi in a more natural and healthy way. Therefore the present study was conducted to identify the antifungal effect of commonly available plant products against *Aspergillus parasiticus* (IMTECH 2797).

MATERIALS AND METHODS

Test organism and culture media: A. parasiticus (IMTECH 2797) was procured from Institute of Microbial Technology, Chandigarh, India. Fungi were subcultured in potato dextrose agar and the slants were stored in refrigerator. For determination of anti-fungal activity, the fungi were harvested from stock culture with distilled water, serially diluted and

the countable number of colonies was identified by spread plate technique. For the whole study, potato dextrose agar (PDA) was used as medium and the growth period was 5 days.

Preparation of plant products: Commonly available plant products (62) belonging to 38 families were identified botanically and used. The products tested included spices, oils, pulp of fresh vegetables, fresh leaves, roots, seeds and tubers (Table 1). Fresh leaves (1.0g) were passed over heat using the bursen burner, similar to the way they are prepared traditionally. The leaves were then squeezed and the resulting juice was used (Akinsulire *et al.* 2007). Fresh vegetables were homogenized, filtered over muslin cloth and the juice was used. The spices and dried herbal products were finely powdered before use. All the products were added at the concentration of 1.0% to the media.

Screening and determination of antifungal effect of plant products: To PDA, 60.0ml of water and the respective plant products were added, autoclaved and the agar with the test compound was poured to petri plates in triplicate. The agar without the test compound served as the control. To these plates, 0.1 ml diluted spore suspension of *Aspergillus parasiticus* (IMTECH 2797) was added, spread and incubated at 27–30°C for 5 days. On day 5, colony counting was done to judge the anti-fungal properties of the compounds. All the experiments were done in triplicate. The mean of observations with standard deviation of the colony forming unit (CFU) under each test compound were calculated.

Table 1. List of plant products used for the study

Botanical name	Family	Botanical name	Family
Syzygium aromaticum	Myrtaceae	*Ficus benghalensis*	Moraceae
Cymbopogon citratus	Poaceae	*Mangifera indica*	Anacardiaceae
Cymbopogan martini	Gramineae	*Bougainvillea glabra*	Nyctaginaceae
Azadirachta indica	Meliaceae	*Tabernaemontana coronaria 'Plena'*	Apocynaceae
Olea europaea	Oleaceae	*Jasminum grandiflorum*	Oleaceae
Brassica juncea	Brassicaceae	*Murraya koenigii*	Rutaceae
Linum usitatissimum	Linaceae	*Ervatamia coronaria*	Apocynaceae
Ricinus communis	Euphorbiaceae	*Calendula officinalis*	Asteraceae
Capsicum frutescens	Solanaceae	*Tinospora cordifolia*	Menispermaceae
Raphanus sativus	Brassicaceae	*Solanum torvum*	Solanaceae
Dolichos lablab	Fabaceae	*Nerium oleander*	Apocynaceae
Solanum melongena	Solanaceae	*Piper betel*	Piperaceae
Phaseolus lunatus	Fabaceae	*Carica papaya*	Caricaceae
Momordica charantia	Cucurbitaceae	*Leucas aspera*	Lamiaceae
Tamarindus indica	Leguminosae	*Couroupita guianensis*	Lecythidaceae
Azadirachta indica	Meliaceae	*Andrographis paniculata*	Acanthaceae
Cynodon dactylon	Poaceae	*Trigonella foenum graecum*	Fabaceae
Citrus limonum	Rutaceae	*Curcuma longa*	Zingiberaceae
Ocimum sanctum	Lamiaceae	*Piper nigrum*	Piperaceae
Coriandrum sativum	Umbelliferae	*Terminalia chebula*	Combretaceae
Amaranthus retroflexus	Amaranthaceae	*Cuminum cyminum*	Umbelliferae
Amaranthus viridis	Amaranthaceae	*Myristica fragrans*	Myristicaceae
Sesbania grandiflora	Fabaceae	*Withania somnifera*	Solanaceae
Mentha piperita	Labiatae	*Glycyrrhiza glabra*	Fabaceae
Punica granatum	Punicaceae	*Papaver somniferum*	Papaveraceae
Psidium guajava	Myrtaceae	*Hemidesmus indicus*	Asclepiadaceae
Hibiscus rosasinensis	Malvaceae	*Quercus infectoria*	Fagaceae
Musa paradisiaca	Musaceae	*Sesamum indicum*	Pedaliaceae
Lawsonia inermis	Lythraceae	*Brassica juncea*	Brassicaceae
Ficus religiosa	Moraceae	*Zingiber officinale*	Zingiberaceae

RESULTS AND DISCUSSION

Fungal infection of feeds should be prevented before any fungal infestation occurs or during the period of fungal invasion of plant material and mycotoxin production as the possibility of infection starts right from field to feeding of animals. Further, if mycotoxins are produced, it is difficult to eliminate them in a practical way (Jouany 2007). Today, mostly chemical fumigants or chemical-based protective agents are used for the safe preservation of fresh produce. The major drawback with such synthetic compounds is the carryover of the toxic residues to both animals and humans. Some of these chemicals are either banned in developed countries or are likely to be banned in developing countries. Hence in the recent years, more emphasis is on search for antifungal materials from natural sources (Reddy *et al.* 2010). Oils and extracts of plants proved to be useful in control of pre-and post-harvest fungal diseases (Singh and Maurya 2005).

The plant products exhibited varying effects against *A. parasiticus*. To determine the anti-fungal effect, the percentage reduction in growth was considered. In addition, compounds which produced tiny colonies (diameter <0.4 cm) and colonies without spores were also recorded. The colony count and percentage inhibition by plant products are given in Fig.1.

Among the products screened, *Syzygium aromaticum* (clove) oil exhibited maximum inhibition (98.86%, Fig.1). This was followed by *Cymbopogan citratus* (lemongrass) oil (41%) that produced tiny colonies without spores.

C. martini inhibited *Aspergillu*s growth by 7.5% (Fig.1) while the oils of *Azadirachta indica, Olea europaea, Brassica juncea, Linum usitatissimum* and *Ricinus communis* did not show any fungicidal effect against the fungi studied.

Syzygium aromaticum is widely cultivated in several countries and possess anti-fungal activity against yeasts and several food-borne fungal species (Pinto *et al*. 2009). The antifungal effect of *S. aromaticum* oil observed in the present study was reported earlier against *A.parasiticus* (NRRL 2999)

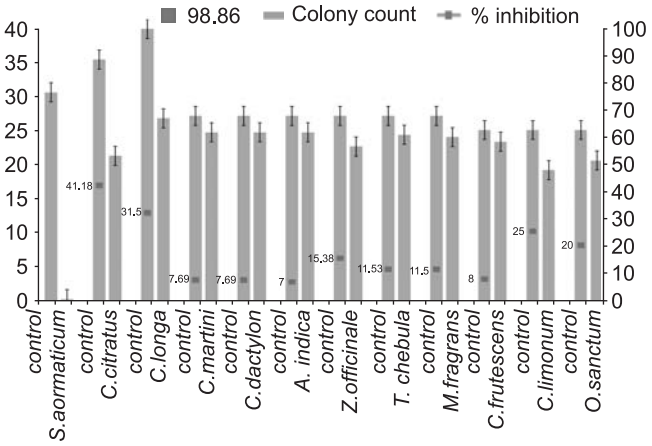

Fig. 1. Antifungal effect of plant products against *A. parasiticus.*

(Gowda *et al*. 2004). Further, *S.aromaticum* oil showed strong antifungal activity against *Aspergillus fumigatus*, and the essential ingredient responsible for its antifungal activity is eugenol (Rana *et al*. 2011). The antimicrobial activity of eugenol is higher against fungi than bacteria. *S. aromaticum* oil is suggested as a biopreservative for foods. Consumption of food products with the oil as a natural preservative is not harmful to human health and environment (Gupta *et al*. 2009).

Cymbopogon citratus, a spice plant commonly known as lemongrass, is of West Indian origin and yields an essential oil with high content of citral (>70%, Paranagama *et al*. 2003). Besides *A. parasiticus* (IMTECH 2797), *C. citratus* oil is effective against *A. flavus* (Agboola *et al*. 2012), *A. niger* (Helal *et al*. 2006) and *A. fumigatus* (Khan and Ahmad 2011). The secondary metabolites of the plants serve as important source of microbicides, pesticides and many pharmaceutical drugs (Billerberk *et al*. 2001). Citral is suggested as one of the most important biological active compounds with fungicidal properties in *C. citratus* (Elagayyar *et al*. 2001). *C.citratus* oil caused morphological changes in *A. parasiticus* (Shaaban *et al*. 2010). In the present investigation, absence of sporulation and reduction in colony size were observed. In this regard, Rasooli and Abyaneh (2004) indicated morphological changes including lack of sporulation, loss of pigmentation, aberrant development of conidiophores and distortion of hyphaes in *Aspergillus* fungi exposed to essential oils. The lipopilic nature of oils aid in penetration of plasma membrane and rupture of plasmalemma of fungal cells (Shaaban *et al*. 2010).

Fresh leaves of *Azadirachta indica, Cyanodon dactylon, Citrus limonum* and *Ocimum sanctum* inhibited fungal growth by 7–20% (Fig.1). The leaves of *Coriandrum sativum, Amaranthus retroflexus, Amaranthus viridis, Sesbania grandiflora, Mentha piperita, Punica granatum, Psidium guajava, Hibiscus rosasinensis, Musa paradisiaca, Lawsonia inermis, Ficus religiosa, Ficus benghalensis, Mangifera indica, Bougainvillea glabra,Tabarnaemontana coronaria, Jasminium grandiflorum, Murraya koenigii, Ervatamia coronaria, Calendulla officinalis, Tinospora cordifolia, Solanum torvum, Nerium oleander, Piper betel, Carica papaya, Leucas aspera, Courapita guianensis, Andrographis paniculata* and *Trigonella foenum* did not inhibit growth of *A. parasiticus.* The pulp of *Zingiber officinale* and *Capsicum frutescens* inhibited fungal growth by 7–15% (Fig.1) whereas *Raphanus sativus*, *Dolichos lablab, Solanum melongana, Phaseolus lunatus, Momordica charantia* and *Tamarindus indica* did not show any fungicidal effect.

Supplementation of *Curcuma longa* and *Piper nigrum* reduced the colony size and inhibited spore production in *A. parasiticus* whereas *Terminalia chebula* and *Myristica fragrans* inhibited fungal growth by 7–11%. *Cuminum cyminum* did not affect the vegetative growth but the colonies grown in *C. cyminum* supplemented medium were devoid of spores. No anti-fungal effect was exhibited by dry powders

of *Trigonella foenum*, *Withania somnifera, Glycyrrhiza glabra, Papaver somniferum, Hemidesmus indicus, Quercus infectoria, Zingiber officinale, Sesamum indicum* and *Brassica juncea.*

The oil of *C. longa* exhibited moderate anti-fungal effect against *Aspergillus fumigatus* and *Aspergillus niger* (Singh and Maurya 2005). Essential oil of turmeric leaves (*Curcuma longa*) significantly inhibited the growth of *Aspergillus flavus* and production of aflatoxin B1 and G1 (Sindhu *et al*. 2011). The study by Jahanshiri *et al.* (2012) indicated an inhibition of fungal growth, toxin production and expression of *ver-1*, *nor-1*, *pksA*, *omtA* and *aflR* genes that are involved in aflatoxin biosynthesis in *A. parasiticus* (NRRL 2999) by curcumin. The essential oil of *O. sanctum* inhibits the growth of *A. flavus* (Kumar *et al*. 2010) and ethylacetate extract of *A. paniculata* inhibits *A. niger* growth (Sule *et al*. 2012). However in the current study the effect of *O. sanctum* leaves was minimum. The antifungal effect of *Z. officinale* oil is moderate against *A. flavus* (Agboola *et al*. 2012) whereas the effect of tubers is minimum against *A. parasiticus*. This could be due to the presence of active fungicidal components in the oil. The natural products are suggested to act by granulation of cytoplasm, rupture of cytoplasmic membrane and inactivation and/or inhibition of synthesis of intra- and extra-cellular enzymes and thereby exert the fungicidal effects (Souza *et al*. 2005).

In conclusion, the present study indicated that *S. aromaticum, C. citratius* and C. *longa* are effective in inhibiting the growth of *Aspergillus parasiticus* (2797). Identification of the active components and their modes of action would help to exploit the products for the benefit of the feed manufacturers, farmers and the livestock industry as a whole.

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