Int. J. Biol. Res., 2(2): 89-92, 2014.

OBSERVATION OF SEED BORNE MYCOFLORA RELATED WITH CUMIN (*CUMINUM CYMINUM* L.)

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ABSTRACT

Cuminum cyminum L. (cumin), herbaceous, annual and medicinal plant belongs to the family Apiaceae. Seeds of cumin were collected from different localities of Pakistan for the observation of mycoflora. Twenty one fungal species were isolated from 12 samples of cumin by using ISTA (International Seed Testing Association) techniques. Blotter method yielded maximum number of fungi followed by deep freezing and agar plate method. *Aspergillus* species were dominant saprophytic fungi while from pathogenic fungus, *Fusarium oxysporum* was prominent one.

KEYWORDS: Cumin seeds, ISTA technique, Pathogenic fungi, Seed borne mycoflora, Calcium hypochlorite.

INTRODUCTION

Cumin (*Cuminum cyminum* L.) considered to be a type of spice crop belongs to family Apiaceae. Commonly, it is called 'zeera' and it is most important export crops of some Asian countries (Kafie *et al*., 2002; El-sawi and Mohamed, 2002). According to Aminpoor and Mousavi (1997); Li and Jiang (2004), paranshimic organs of cumin have oils, resins and monoterpens. Seeds contain 3 to 4% volatile oil and approximately 15% of fixed oil (Zarghari, 1982). Essential oil of cumin contains aldehyde (*p*-isopropyl-benzaldehyde, 25 to 35%), perilla aldehyde, cumin alcohol, α- and β- pinene (21%), dipentene, *p*-cymene and β-phellandrene (Avatar *et al*., 1991; El-sawi and Mohamed, 2002; Li and Jiang, 2004). According to Romeilah *et al.* (2010) *C. cyminum* is used as preservative, stimulant, antspasmodic and also have an antimicrobial activities. Also used as curry powder, pickle and chutney (Farrell, 1985).

Variety of fungal species have been reported from cumin seeds including *Aspergillus flavus, A. niger, A. fumigates, A. ochraceus, A. candidus, A. sydowii, Chaetomium dolicholrichum, Fusarium* species, *Alternaria* species, *Curvularia* and *Rhizopus* species (Roy *et al.,* 1988). Elshafie *et al*. (2002) collected five samples of seven spices i.e., cumin (*Cuminum cyminum* (L), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), black pepper (*Piper nigrum)*, cardamom (*Elettaria cardamomum)*, ginger (*Zingiber officinale)*, and coriander (*Coriandrum savitum* L.) from five popular companies in the Sultanate of Oman for the observation of the mycoflora and aflatoxins. Twenty fungal species were isolated in which *Aspergillus flavus*, *A. niger*, *Penicillium*, *Rhizopus*, and *Syncephalastrum racemosum* were the most dominant.

Due to its economic importance in Pakistan, cumin seeds have been selected for the study of associated mycoflora.

MATERIALS AND METHODS

Collection of seed samples: Twelve samples of cumin were collected from Karachi (6), Sargodha (1), Raheem yar Khan (1), Sukkur (1), Jhung (1), Multan (1), Peshawar (1). 400 seeds of each sample were used in each technique.

Determination of seed borne fungi: Seed borne mycoflora were observed by using ISTA (International Seed Testing Association) techniques which included Standard blotter method, Agar plate method and Deep-freezing method (Anon., 1993).

i. Standard blotter method: Non surface disinfected and seeds after surface disinfected with 1% $Ca(OCl)_2$, placed on three layers of moistened blotter paper with 10 seeds per Petri dish. The dishes were incubated for 5-7 days at 28 ± 2°C under 12 h, alternating cycle of artificial day light (ADL) and darkness.

ii. Agar plate method: Non surface sterilized and seeds after surface disinfected with 1% $Ca(OCl)_2$, placed aseptically on sterile Potato dextrose agar (PDA), and were incubated for 5-7 days at 28 ± 2°C under 12 h, alternating cycle of artificial day light (ADL) and darkness.

iii. Deep-freezing method: Non surface sterilized and seeds after surface sterilization with 1% $Ca(OCl)_2$, placed aseptically on three layers of moistened blotter paper. Ten seeds per Petri dish were incubated for 24 h at 28 ± 2°C and then at –2°C. Finally they were incubated for 5 days at 28 ± 2°C under 12 h alternating cycle of artificial day light (ADL) and darkness.

Identification of mycoflora: Identification was done by using different mycological literature (Barnett and Hunter, 1998; Booth, 1971; Ellis, 1971; Gilman, 1950; Nelson *et al.,* 1983; Raper *et al.,* 1965; Domsch *et al.,* 1980).

Data analysis: Analysis of mycoflora was done following the procedure given by Sokal and Rohlf (1995).

RESULTS AND DISCUSSION

Twenty one fungal species of 10 genera were isolated from cumin seeds collected from different localities of Pakistan. The fungi observed in 12 samples were *Absidia corymbifera* (Cohn) Sacc. & Trotter, *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire, *Alternaria* sp., *A. longipes, Aspergillus clavatus* Desm., *A. flavus* Link ex Gray, *A. flavipes* (Bain. & Sart.) Thom & Church, *A. niger* Van Tieghem, *A. ochracheus* Wilhelm, *A. ustus* (Bain.) Thom & church, *A. wentii* Wehmer, *A. tamarii, Cladosporium* spp Lik. Fr, *Fusarium oxysporum* Schlecht, *F. solani* W.C. Snyder & H.N. Hansen, *Mucor* spp. Mich. Ex St.-Am, *Paecilomyces variotii, penicillium* sp., *Rhizopus* spp. Went & prinsen Geerligs, *Trichoderma harzianum* Pers. Ex. Fr., *Trichoderma viride* Pers. Ex. Fr. etc. Of this 16 species belonging to 8 genera were isolated from blotter method, 15 species of 7 genera from agar plate method while deep freezing method yielded 15 species belonging to 8 genera (Table 1). *A. flavus* followed by *A. niger* was the dominant fungi which were isolated from almost all the samples ($p<0.001$). *Absidia corymbifera* and *A. tamarii* were isolated from only one sample by agar plate method. Kulshrestha *et al.* (2014) observed that *A. niger* was in maximum count while *A. alternata, Emericella nidulans, Fusarium oxysporum, Penicillium chrysogenum, P. citrinum, P. notatum, Rhizopus arrihizus* and *R. stolonifer* was recorded in minimum incidence. Ayres *et al.* (1980); Takatori *et al.* (1977) reported that *Aspergillus* and *Penicillium* species were the main components of spices like cumin, cardomom, Cinnamon, fennel and coriander.

Surface sterilization of cumin seeds by calcium hypochlorite significantly ($p<0.001$) reduced the number of saprophytic fungi like of *A. tamari, A. ochracheus, A.ustus* and *A. niger*. Several researchers observed similar results on different seeds (Rahim *et al.,* 2013; Niaz and Dawar, 2009; Tariq *et al.,* 2005; Rahim and Dawar, 2012). Other reason could be that the reduction in fast growing fungi, giving opportunity for the growth of deep seated fungi. Of the three methods used, blotter method yielded highest number of fungi followed by deep-freezing and agar plate methods. Elwakil and Ghoneem (1999) recorded similar results on black cumin due to quick growth of saprophytic fungi. *T. harzianum* was isolated from agar plate and blotter method while *T. viride* from all the three methods. *A. clavatus, A. flavus, A. flavipes, A. niger, A. wentii, F. oxysporum, Mucor* spp. and *P. variotii* were isolated from all the three methods used.

Lacking in proper post harvest preservation techniques, yield of cumin was damaged due to fungal activities. Care should be taken in processing, storage and transport of cumin seeds to avoid fungal contamination and spoilage.

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(Received March 2014; Accepted July 2014)