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SURVEY ON SEED BORNE FUNGI OF *PUNICA GRANATUM* L. (POMEGRANATE) SEEDS

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ABSTRACT

International Seed Testing Association (ISTA) technique was used for the isolation of seed borne mycoflora. Twenty three fungal species belonging to 11 genera from 12 seed samples of *Punica granatum* L. (pomegranate) collected from various localities of Pakistan. Agar plate method was found to be the best for the isolation of fungi followed by deep freezing and blotter method. By using agar plate method, 16 species with 08 genera were isolated while 15 species belonging to 07 genera were isolated by deep freezing and 15 species with 06 genera by blotter method. *Aspergillus clavatus, A. flavus, A. flavipes, A. niger, A. ustus, A. wentii, Mucor* spp., and *Paecilomyces variotii* were isolated from all the methods. Seeds of *P. granatum*when surface sterilized with 1% Ca $(OCl)_2$ was found to be less effective against fungi.

INTRODUCTION

Pomegranate seeds (*Punica granatum* L.) belongs to the family Punicaceae and is commonly known as "Anardana", originating from the Middle East, extending throughout the Mediterranean, eastward to China and India and on to the American Southwest, California and Mexico (Fink & Cookson, 2007). The name pomegranate originates from the genus '*Punica*', which was the Roman name for Carthage, where the best pomegranates were known to. It is known by the French as grenade, the Spanish as Granada, which literally translates to seeded ('*granatus*') apple ('*pomum'*) (Langley, 2000). It is cultivated in Pakistan on an area of 12.9 thousand hectares and the production of 50.0 thousand tones (Anon, 2011).

Pomegranate is one of the oldest edible fruit has a long history as a medicinal fruit and has been extensively in the folk of many cultures (Longtin, 2003). *P. granatum* is a dwarf variety with its numerous small flowers and long flowering period (Jianzhu *et al.*, 2003). The edible part of the fruits contains acids, sugars, vitamins, polysaccharides, polyphenols and minerals. Recent study showed high contents of vitamin C in different pomegranate varieties ranging between 312 to 1050 mg/100 g (Dumlu & Gurkan, 2007). Constituents of *P. granatum* includes gallocatechins, delphinidin, cyaniding, gallic acid, ellagic acid, pelargonidin and sitosterol which are very well known for their therapeutic properties (Singh *et al.*, 2002).

As far as knowledge is concerned about the presence of fungal flora on pomegranate seeds was unavailable. Due to its nutritional values and economical importance, seeds of pomegranate were collected from different cities of Pakistan and observe mycoflora present on it by using ISTA technique.

MATERIALS AND METHODS

Collection of seeds: Twelve Pomegranate seed samples were collected from the markets of different localities of Pakistan viz., Karachi (6), Sargodha (1), Raheemyar Khan (1), Sukkur (1), Jhung (1), Multan (1), Peshawar (1).

Detection of mycoflora: For the detection of seed-borne mycoflora, ISTA techniques were used (Anon, 1993). By using standard blotter method, agar plate method and deep-freezing methods, 400 seeds of each sample were tested.

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

b. Agar plate method: Untreated and seeds after treatment with 1% Ca $(OCl)_2$ for 2 minutes were placed on Potato dextrose agar (PDA), 10 seeds per Petri dish. The dishes were incubated for 5-7 days at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness.

c. Deep-freezing method: Untreated and seeds after treatment with 1% Ca $(OCl)_2$ for 2 minutes were placed on three layers of moistened blotter paper, 10 seeds per Petri dish were incubated for 24h, each at 28±2°C and -2°C followed by 5 days incubation at 28±2°C under 12h, alternating cycle of artificial day light (ADL) and darkness.

Identification of fungi: Mycoflora growing on seeds were identified after referencing to Barnett (1960), Domsch *et al*., (1980), Ellis (1971), Nelson *et al*., (1983), Raper & Fennell (1965), Booth (1971).

Data analysis: Data was subjected to analysis of variance (ANOVA) following the procedures as suggested by Gomez & Gomez (1984).

RESULTS

Twenty three fungal species belonging to 11 genera viz., *Alternaria tenuissima* (Kunze ex pers.) Wiltshire., *Aspergillus clavatus* Desm., *A. candidus* Link ex Link, *A. flavus* Link ex Gray., *A. fumigatus* Fres., *A. flavipes* (Bain. & Sart.) Thom & Church, *A. niger* Van Tieghem., *A. ochracheus* Wilhelm, *A. ristrictus* G Sm., *A. terreus* Thom, *A. ustus* (Bain.) Thom & church, *A. wentii* Wehmer, *Cladosporium cladosporioides* (Fres.) de Vries., *Drechslera australiensis* (Bugnicourt) Subram. & Jain ex M.B. Ellis, *Fusarium oxysporum* Schlecht. Emend. Sny. & Hans., *F. solani* W.C. Synder & H.N. Hansen, *Geotricum candidum* Link, *Mucor hiemalis* Wehmer, *Paecilomyces variotii* Samson, *Penicillium* spp., Link ex Fr., *Rhizopus stolonifer* (Ehrenb. Ex Link) Lind, *Trichoderma harzianum* Rifai, *T. viride* Pers. ex Gray were isolated from the seed samples collected from various localities of Pakistan (Table 1). Agar plate method was found to be more suitable for the isolation of fungi followed by deep freezing and blotter methods where agar plate method yielded 16 species belonging to 08 genera, deep freezing method showed 15 species with 7 genera while blotter method yielded 15 fungal species belonging to 06 genera. Pathogenic fungi like *Fusarium oxysporum, F. solani* and *G. candidum* were only recorded from deep freezing method, causing rot and decay of seeds and seedlings. Species of *Aspergillus* particularly *A. clavatus, A. flavus, A. flavipes, A. niger, A. ustus* and *A. wentii* were recorded from all sterilized and non sterilized seeds accounted for ISTA technique which caused complete rottening of seeds. Significant ($p<0.001$) appearance of *A. niger* and *A. flavus* were recorded on all 12 samples of pomegranate seeds except for *A. flavus* on deep freezing method which showed its appearance on 9 samples. Seeds surface sterilized with 1% Ca $(OCl)_2$ showed reduction of saprophytic fungi as compared to non surface sterilized seeds. Antagonistic fungi namely *T. viride, T. harzianum* and *P. variotii* were also observed out of which, *T. harzianum* was recorded from sterilized and non sterilized blotter method while *P. variotii* was recorded from all the three methods.

DISCUSSION

Pomegranate seeds showed presence of 23 fungal species of which majority of fungi were isolated on agar plate method. According to Neergaard (1977), PDA method was most sensitive in detection of even traces of fungal infection and intra fungal antagonism become problem in agar plate method (Limonard, 1968). Rahim *et al*., 2010; Kumar *et al.,* 2002; Hussain *et al*., 2007) on lentil observed that agar plate method was most suitable in detection of mycoflora. Deep freezing method showed maximum percentage of pathogenic fungi after sterilization with Ca $(OCl)_2$. *C. cladosporioides*, *F. oxysporum, F. solani, G. candidum* were isolated from deep freezing method. Mathur *et al.,* (1975); Niaz & Dawar (2009) recorded that deep freezing method was significant in isolation of pathogenic fungi. *Fusarium* species caused wilting and plants becomes yellowed leaves with minimal or very much reduced crop yield. The severity of fungus results in killing of primary and secondary roots (Alabouvette *et al.,* 1993). Calcium hypochlorite was reported to reduce the fast growing saprophytic and mold fungi. Same results were observed by Dawar & Ghaffar (1991) on sunflower; Tariq *et al.,* (2005) on soy bean; Kumar *et al.,* (2002) on lentil. Species of *Aspergillus* particularly, *A. flavus* which is responsible for aflatoxin production was recorded from all the samples tested. However, aflatoxins are responsible for the production of aspergillosis and systemic infections in man, animals and birds (Raper & Fennell, 1965). *A. niger* produced two carcinogenic mycotoxins namely fumonisins and ochratoxins. Frisvad *et al*., (2011) observed thatfumonisins (B_2, B_4, B_6) was detected in 81% of *A. niger* and ochratoxin A in 17% while 10% strains produced both toxins. Tannins, which are water soluble polyphenols, present in peels of pomegranate fruits, was effective in reduction of *A. niger* due to the fact of inhibition of oxidative phosphorylation pathway (Scalbort, 1991).Two antagonistic fungi viz., *Trichoderma* species and *P. variotii* were also reported on pomegranate seeds, of which *P. variotii* was isolated from all the three methods used while *T. harzianum* and *T. viride* from agar plate and blotter methods respectively. *Trichoderma* species are free living fungi which are present in roots, soil and foliar environments and they are known to produce variety of antibiotic substances and their capability to parasitize other fungi (Sivasithamparam & Ghisalberti, 1998). Major steps should be taken for the reduction of pathogenic and mould fungi by improving the storage condition.

References

Alabouvette, C., P. Lemanceau and C. Steinberg. 1993. Recent advances in the biological control of *Fusarium* wilts. *Pesticide Science,* 37(4): 365-373.

Anonymous. 2011. *Agricultural Statistics of Pakistan.* Ministry of Food, Agriculture & Livestock. Economic Wing, Govt. of Pakistan, Islamabad. pp. 99.

Anonymous.1993. International Rules for Seed Health Testing. *Seed Science & Technol*., 21: 1-288.
Barnett, H.L. 1960. *Illustrated genera of imperfect fungi* (second edition). Burgess Pub., Co., pp. 225.
Booth, C. 1971. *The genus Fusarium*. CMI, Kew, Survey, England. pp. 237.
Dawar, S. and A. Ghaffar. 1991. Detection of seed borne mycoflora of sunflower. *Pak. J. Bot.*, 23: 173-178.
Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of Soil Fungi.* Vol. 1, London: Academic Press.
Dumlu, M.U. and E. Gurkan. 2007. Elemental and nutritional analysis of *Punica granatum* from Turkey. *Journal of Medicinal Food*, 10(2): 392-395.
Ellis, M.B. 1971. *Dematiaceous Hyphomycetes.*CMI, Kew, Surrey, England. pp. 608.
Fink, S.L. and B.T. Cookson. 2007. Pyroptosis and host cell death responses during *Salmonella* infection. *Cell Microbial*, 9: 2562-70.
Frisvad, J.C., T.O. Larsen, U. Thrane, M. Meijer, J. Varga, R.A. Samson and K.F. Nielsen. 2011. Fumonisin and ochratoxin production in Industrial *Aspergillus niger* strains. *PLOS One*, 6(8): 1-6.
Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for Agricultural Research. 2nd ed. Willy, New York.
Hussain, M.A., T. Mukhtar, M. Irfanul-Haque and M.Z. Kayani. 2007. Mycoflora associated with lentil *Lens esculenta* (Moench) seeds from five localities of Punjab, Pakistan. *Pak. J. Bot.,* 39(3): 903-906.
Jianzhu, S., C. Chunli and D. Xiuxin. 2003. *In-vitro* induction of tetraploid in pomegranate (*Punica granatum*). National Key Laboratory of Crop Genetic Improvement. *Plant Cell Tissue and Organ Culture*, 75: 241-24.
Kumar, D.R., R.P. Dwivedi and N. Udit. 2002. Studies on the seed borne fungi of lentil. *Annals of Plant Protection Sciences*, 10(1): 114-117.
Langley, P. 2000. Why a pomegranate? *BMJ*, 321: 1153-1154.
Limonard, T. 1968. *Ecological aspects of seeds health testing. International Seed Testing Association*, *Wagoningen, Netherland*. 167pp.
Longtin, R. 2003. The pomegranate: Nature's power fruit. *Journal of the National Cancer Institute*, 95: 346-348 pp.
Mathur, S.K., S.B. Mathur and P. Neergaard. 1975. Detection of seed borne fungi in sorghum and location of *Fusarium moniliforme* in seed. *Seed Sci. & Technol.*, 13: 683-690.
Neergaard, P. 1977. Seed Pathology. MacMillan, Press Ltd., London and Basinstoke. Vol. 2, pp. 1187.
Nelson, P.E., T.A. Toussoun and W.F.O. Marasas. 1983. *Fusarium* species. *An Illustrated Manual of Identification.* University Park, Pennsylvania: The State University Press.
Niaz, I. and S. Dawar. 2009. Detection of seed borne mycoflora in maize (*Zea mays* L.). *Pak. J. Bot*., 41(1): 443-451.
Rahim, S, S. Dawar and M. Tariq. 2010. Mycoflora associated with lentil (*Lens culinaris* L.) seeds of Pakistan. *Pak. J. Bot*., 42(6): 4345-4352.
Raper, K.B. and D.I. Fennell. 1965. The genus *Aspergillus.* The William & Wilkins company, Baltimore. pp. 686.
Scalbert, A. 1991.Antimicrobial properties of tannins. *Phytochemistry*, 30: 3875-3883.
Singh, R.P., M.K.N. Chidambaram and G.K. Jayaprakasha. 2002 Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *In vitro* models. *J. Agric. Food Chem*., 50: 81-6.
Sivasithamparam, K. and E.L. Ghisalberti. 1998. *Secondary metabolism in Trichoderma and Gliocladium.* Vol. 1. Taylor and Francis Ltd., London, pp. 139-191.
Tariq, M., S. Dawar, M. Abid and S.S. Shaukat. 2005. Seed-borne mycoflora of soybean. *Int. J. Biol. Biotech.*, 2: 711-713.

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