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Mycodiversity of Seed Borne Flora of Rose Balsam (*Impatiens balsamina* L.)

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Abstract:

The mycological analysis of seed borne fungal flora of composite seed samples of Rosa balsam (*Impatiens balsamina* L.) from different geographical regions of Nagpur has revealed prevalence of 23 fungal species belonging to 15 genera. Altogether, a population of 20 fungal species representing 13 genera has been encountered from untreated seeds on blotter paper as external seed borne while 14 fungal species of 9 genera from treated seeds on agar plate as internal seed borne. *Aspergillus amstelodomi* was recorded most dominant as external seed borne while *Aspergillus flavus* was confined dominant among internal seed borne fungal flora. Deuteromycota contributed higher level of fungal incidence and fungal count. Maximum fungal flora was detected on blotter paper over agar plate.

Keyword:
Impatiens balsamina L., seed borne, fungal flora, dominant, population.

Introduction:

Impatiens balsamina L. (Gulmendi), an annual herb of family Balsaminaceae is native to southern Asia in India, Myanmar and Burma but presently grown extensively worldwide as ornamental plant in response to its use in Ayurvedic medicine as plant naturally produces a wide variety of secondary metabolites which have a good antibacterial, antifungal, anti-pests effect (Wikipedia, 2014). The whole plant extracts induce a long lasting skin moisturizing effect and prevent dryness, rough skin chap, dandruff and splitting hair ends, hence are used to prepare lotions, creams, hair tonics, cosmetics, bath preparations and detergents (Toki *et al.*, 2000). Alcoholic extract of the flowers has been reported to be useful for pains in the joints. Roots are used to treat jaundice and digestive disorders. Juice from balsams leaves treats warts and also snakebite, while the flower can be applied to burns to cool the skin. The plant has been used for the treatment of thorn or glass-puncture wounds, abscesses, in grown nails and chronic ulcers caused by allergic reaction of detergents (Rajasekaran et al., 2009). A population of microbes including fungal and bacterial species has been confined to this plant (Rajendran et al., 2014). So far seed borne fungal flora from this plant was not reported hence the study was undertaken to investigate the fungal flora associated with seeds of *Impatiens balsamina* L.

Material and methods:

A composite seed sample of *Impatiens balsamina* from different regions of Nagpur has been screened for prevalence of apparent deformities or discoloration by dry examination method. The isolation of seed mycoflora was made from infected seeds employing standard blotter and agar plate method (ISTA, 2014). The colonies developed on the untreated and pre-treated seeds were counted, isolated and identified after sub-culturing on Czapek's nutrient media in tube slants. The species were identified on the basis of micro- and macro morphology; reverse and surface coloration of colonies and finally authenticated by authority. Fungal count and infestation level have been recorded as a percentage of infested seeds in a sample following a technique reported earlier (Chukunda et al., 2013). Purified fungal isolates were propagated and maintained on Czapek's Dox agar nutrient medium in sterile slants.

Results and discussion:

Viable healthy seeds may act as catalyst for realizing the potential of all other inputs. Such seeds can be affected by pathogenic fungal contaminants or through contaminated seeds which may fail to germinate, cause infection to seedlings and reduce the global productivity (Chukunda et al., 2013). The blotter paper and agar plate technique recommended for seed health testing and standardized time to time by ISTA (2014) for accuracy are applied in for detection of seed-borne fungal flora as these two tests are inevitable for getting a complete picture of the fungal infection/association with seeds (Saskatchewan, 2013).

Mycological analysis of a composite seed sample of *Impatiens balsamina* L. revealed prevalence of population of total 23 fungal pathogens of diverse groups, categorized under 15 genera in varying incidence (Table 1). Of the total fungal count, 11 isolates representing 7 genera has been isolated as both external and internal seed borne; 3 isolates belonging to 2 genera confined only as internal seed borne while 9 genera representing 7 genera as external seed-borne. Deuteromycota dominated with highest 47.8% fungal count followed by Ascomycota (30.3%), Zygomycota (13.0%). Oomycota had least count of isolates. Fungal spores from Basidiomycota did not persist on the seed surface. *Fusarium* dominated with a higher count of 4 species; *Aspergillus* and *Curvularia* with three species; two species contributed by *Cladosporium* while remainings had single species (Table 1).

The significant level incidence was confined to seed of *Impatiens balsamina*. Deuteromycota dominated with 43.0% incidence followed by Ascomycota (41.1%) and Zygomycota (14.8%) while Oomycota contributed least

incidence. *Aspergilli* dominated with greater incidence; *Fusarium* and *Curvularia* had moderate while others had least level of incidence (Table 1). Amongst members of Deuteromycota, the *Fusarium* had greater incidence while it was significant with *Curvularia,, Colletotrichum, Paecilomyces,* and *Stachybotrys*. Moderate level infestation was recorded for *Botryodiplodia sp., Cunninghamella elegans,* while others had little incidence. In Oomycota, *Phytophthorain festans* had higher incidence over *Pythium* sp. In Zygomycota, *Rhizopus stolonifer* had higher level of infestation. Out of the total, level of infestation to the extent of 54% was recorded on blotter paper while it was confined 45.0% on agar plates. It is in confirmation with findings of Bhajbhuje (2014); Gayatri and Madhuri (2014) who reported higher incidence on infested stored seeds of pulses, tomato and safflower by blotter test Deuteromycota contributed greatest fungal incidence. In this group, *Fusarium* contributed significant higher level of incidence against others. It is in agreement to finding of Kakde *et al.*, (2012) who reported predominant occurrence of Deuteromycetous members on oil seeds.

The efficacy of both standard blotter and agar plate tests varied with nature of fungal flora. Among the seed health test techniques, standard blotter method was proved comparatively superior over agar plate method to the fungal pathogens isolation. Hedawoo *et al.*, (2014) pointed out the quick growing saprophytes adhering to the outer seed coat which may be troublesome to detect internal slow growing pathogen on agar plate. These variations may possibly attribute to the prolonged incubation that might lead to the development of deep seated infection; (Ismail.et al., 2012); and (Bhajbhuje, 2014).

*Aspergilli*and *Cladosporium* of Ascomycota as well as, *Curvularia, Fusarium* and *Paecilomyces* of Deuteromycota contributed as major components on *Impatiens balsamina* L. seeds represented a group of taxa of cosmopolitan fungal organisms that can exploit virtually any organic substrate. Deuteromycota had comparatively higher count of fungal isolates with greater level of incidence followed by Ascomycota (Table 1). It may possibly due to prevalence of greater count of fungal propagules associated with seed coat with their higher incidence. Moreover, members of this group are known facultative parasites on crop plants as well as involved as saprophyte in biodegradation of seeds, and debris of plant and animal origin (Jyoti and Malik, 2013; Bhajbhuje, 2013). Members of Deuteromycota complete their life cycle asexually producing abundant, resistant, thick walled conidia which may remain viable for longer duration in adverse climate (Gayatri and Madhuri, 2014). The conidia of *Cladosporium* and *Curvularia* remained in greatest abundance under storage even at low humidity during warmer climate (Kakde *et al.*, 2012). It was interesting to record that members

of Basidiomycota did not persist on *Impatiens balsamina* L. seeds may be possibly attributed to mode of nutrition as majority of fungal organisms of these groups are obligate parasites of other crop plants.

The report of the present study revealed that *Aspergilli* and *Fusaria* were the highly predominant on *Impatiens balsamina* L. seeds are among the most abundant and widely distributed organisms on the globe (Gayatri and Madhuri, 2014). *Aspergilli* are commonly isolated from seeds, soil, plant litter, dried fruits and nuts (Jyoti and Malik, 2013). *Aspergillus niger* has potential to produce *ochratoxin-A*; *Aspergillus flavus* secretes aflatoxin which proved to be nephrotoxic in pigs and broilers (EFSA, 2011). *Curvularia lunata* produces 2-methyl-(5-hydroxy methyl) furan-2 carboxylate; that have been reported to cause a variety of toxic effects in both experimental animals and in human. *Fusarium solani* and *F. moniliformae* were reported to cause *keratitis* and also associated with wound and infections of the eyes and fingernails (EFSA, 2011).

Mucorpusillus secretes Citrinin and Penetrem- A. All these toxins are known to create physiological disorders to consumers (EFSA, 2011). Majority of fungal isolates involved in seed deterioration of *Impatiens balsamina* L. are xerophilic moulds such as *Aspergilli* and *Penicilli* of Ascomycota as well as *Curvularia, Fusarium* of Deuteromycota (Bhajbhuje, 2014). Planting of deteriorated seeds, increases chances of pathogen transmission to a new crop. The toxic metabolites secretion by these isolates may be one of the reasons to spoilage of stored seeds (Jyoti and Malik, 2013). Mycotoxins alter regular metabolism, induced physiological and biochemical changes in host cells resulting abnormal proliferation of plant cells (Jyoti and Malik, 2013).

Table 1: Per cent incidence of fungal contaminants in storage on *Impatiens balsamina L.* seeds.

S. No	Name of fungal isolates	Frequency (%) of fungal incidence		Total Frequency	% over total incidence	
		Blotter	Agar		Species	Genus
A	**Oomycota**	4.5(1.2)		4.5(1.2)	1.2	1.2
1	*Phytophthorainfestans*de Bary.	2.5 (0.6)	-	2.5 (0.6)	0.6	0.6
2	*Pythiumsp*	2.0 (0.5)	-	2.0 (0.5)	0.5	0.5
B	**Zygomycota**	37.0(9.7)	19.5(5.1)	56.5(14.8)	14.8	14.8
3	*Cunninghamelaelegans* Lender	5.5 (1.4)	-	5.5 (1.4)	1.4	1.4
4	*Mucorpusillus*Lindt.	16.0 (4.2)	8.5 (2.2)	24.5 (6.4)	6.4	6.4
5	*Rhizopusstolonifer*(Ehrarb. Ex.Fr. Lind.	15.5 (4.0)	11.0 (2.9)	26.5 (6.9)	6.9	6.9
C	**Ascomycota**	68.5(17.9)	89.0(23.2)	157.5(41.1)	41.1	41.1
6	*Aspergillusamstelodomi*(Mang) Thom and church	24.0 (6.2)	28.5 (7.4)	52.5(13.7)	13.7	33.2
7	*A. flavus*Link	22.5 (5.9)	26.0 (6.8)	48.5(12.7)	12.7	
8	A. niger Van Tieghen	14.0 (3.6)	12.0 (3.1)	26.0 (6.8)	6.8	
9	*Botryodiplodiasp*	4.5 (1.2)	6.5 (1.7)	11.0 (2.9)	2.9	2.9
10	*Cladosporiumcladosporoides*(Fresen) G.A. de Vries	-	7.5 (1.9)	7.5 (1.9)	1.9	4.1
11	*Cladosporium sp.*	-	8.5 (2.2)	8.5 (2.2)	2.2	
12	*Penicilliumsp*	3.5(0.9)	-	3.5 (0.9)	0.9	0.9
D	**Basidiomycota**	-	-	-	-	-
E	**Deuteromycota**	98.0	66.5	164.5	43.0	43.0

		(25.6)	(17.4)	(43.0)		
13	*Colletotrichumdematium* But. andBisby	-	2.0 (0.5)	2.0 (0.5)	0.5	0.5
14	*Curvularialunata*(Wakker) Boedijn	6.5 (1.7)	-	6.5 (1.7)	1.7	5.7
15	*Curvulariaovoidea*(HirosaandWatan) Munt	8.0 (2.1)	-	8.0 (2.1)	2.1	
16	*Curvulariatetramera*(Mck.) Boe. ex Gilman	7.5 (1.9)	-	7.5 (1.9)	1.9	
17	*Diplodiasp*	9.5 (2.5)	-	9.5 (2.5)	2.5	2.5
18	*Fusariumminiliformae*Sheldom	11.5 (3.0)	9.5(2.5)	21.0 (5.5)	5.5	19.4
19	*Fusariumoxysporum*Schlecht	10.5 (2.7)	-	10.5 (2.7)	2.7	
20	*Fusariumsemitectum*BerkandRav.	12.5 (3.3)	14.0 (3.6)	26.5 (6.9)	6.9	
21	*Fusariumsolani*(Mert.) APP. andWollenw	8.5 (2.2)	8.0 (2.1)	16.5 (4.3)	4.3	
22	*Paecilomycesvariotii*Bainier	19.5 (5.1)	21.5 (5.6)	41.0 10.7)	10.7	10.7
23	*Strichibotrisatra*Corda	4.0 (1.0)	11.5 (3.0)	15.5 (4.0)	4.0	4.0
	Total fungal incidence	208	175	383		
	Per cent total incidence	54.3	45.7	100		

Conclusion:

The results revealed that *Impatiens balsamina* L. Seeds harbor arrays of fungal contamination may be associated with the quality of seeds at the time of storage, environmental factors during pre- and post-harvest stages, moisture content, and ambient relative humidity, temperature of storage environment and duration of seeds. The climate of winter season of Nagpur as well as improper storage condition contributes to make the storage environment extremely supportive for fungal attack on nutrient rich *Impatiens balsamina* L.seeds. In order to neutralize the potential of these fungal microbes surviving as agents of seed borne diseases, the steps must be initiated to develop a strategy to antagonize their growth and survival in this seed commodity. Low temperature results in delayed seed deterioration, and, thereby leads to prolonged viability period. Thus seed storage under ambient temperature and relative humidity without deterioration in quality for a longer period is of immense importance for farmers. The farmers are advised to use improved scientific methods of storage to discourage proliferation of these organisms on seeds.

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