Journal of Innovations in Pharmaceuticals and Biological Sciences
www.jipbs.com

JIPBS

ISSN: 2349-2759

Research article

Seed mycoflora associated with ragi (*Eleusine coracana* (L.) geartin

Sobha Rani I., M. Dorcas*

Department of Botany, Osmania University College for Women, Koti, Hyderabad.

Abstract

Ragi (*Eleusine coracana* (L.) Geartin is millet grown as food crop in the states of Karnataka, Telangana and Andhra pradesh in India and also in Srilanka and South African countries. The grain of Ragi (Finger millet) cannot be destroyed easily by insects and can be stored for a longer time with high nutritive value. Hence an attempt is made to study the mycoflora of the seeds and physico-chemical parameters with the germination percentage for a period of six months. Seeds are treated with fungicides like Bavistin, Mancozeb and Zeneb and their effect is studied. About 25 species belonging to 15 genera were isolated from the control and about 12 species belonging to four genera were isolated from fungicide treated seeds. A decrease in fungal numbers is noticed in fungicide treated seeds. During storage a variation from field to storage fungi is noticed.

Key words: Finger millet, Mycoflora, and Fungicides.

***Corresponding Author: M. Dorcas,** Department of Botany, Osmania University College for Women, Koti, Hyderabad, India.

1. Introduction

Ragi can be cultivated throughout the year if adequate moisture is available. Hence it is not a season bound crop.

Kharif crop is sown during May or June. If the rains are not received in time, the sowing time is postponed up to the end of August. However, sowing should be done early in rain fed areas to avoid moisture stress at critical stage of flowering. In areas, where irrigation facilities are inadequate, the sowing should be done soon after the onset of monsoon. In higher hills of northern India, the optimum time of sowing for finger millet is the first fortnight of June. The rain fed early crop is sown in April or early May.

Rabi crop is sown during September and October. The rain fed *rabi* crop in Karnataka, Tamil Nadu and Andhra Pradesh is sown in September and October. The irrigated crop of finger millet is sown in more than one season in Karnataka, Tamil Nadu and Andhra Pradesh. Irrigated crop planting is done during June – July and the summer irrigated crop by planting in December to January.

Two varieties were taken for the study to evaluate the effect of fungicides. Variety CO 13 is 95 to 100 days (Small duration variety). This variety can be grown throughout the year and drought tolerant. Variety CO 10 is 85 days (Small duration).

Drought tolerant is collected from a National seed Corporation, Hyderabad. These two varieties are taken to study Seed Mycoflora associated with it. Ragi or Nachni is the common name of finger millet in south and Northern parts of India This is one of the most nutritious food and easy to digest. It is rich in Calcium (c), Proteins (p), Iron(i), Fiber(f) and other minerals. In the present study, a review of the ecology of seed borne fungi of Ragi is correlated with the important major nutrients.

Food crops grown on are usually propagated through seeds. Seed play vital role in total biological yield. Seed borne pathogens can cause enormous crop losses; reduction in plant growth and productivity of crop [1]. Interaction between seed and mycoflora is complex and varies in different seeds. Temperature, seed moisture content, harvesting, threshing and storing has a profound effect on seed mycoflora. In the present study mycoflora succession, their effect on seed viability and biochemical changes brought by them, along with fungal numbers and the effect of fungicide on seed borne mycoflora are analyzed periodically. Seed-borne diseases have been found to affect the growth and productivity of crop plants [2].

2. Materials and Methods

Detection of seed borne fungi from selected seeds was done by blotter paper method as recommended by the International Seed Testing Association, [3-6] For the standard blotter method, untreated seeds and seeds after treatment with 0.1% Sodium hypochlorite for 10 minutes, seeds treated with fungicides Bavastin, Mancozeb and Zeneb were placed on three layers of moistened blotter, 20 seeds per Petri dish [7]. The plates were then incubated in B.O.D incubator at 25± 2˚C for 8 days under 12 hrs alternating cycles of light and darkness. Thus, the exposed seeds were examined under a microscope for the presence of seed borne fungi. The isolated fungi were identified with the help of the keys, monograph and literature [8-10]. Untreated seeds were used as controls. All experiments were carried out in three replicas.

3. Results and Discussion

Good and healthy seeds are free from seed-borne pathogens and have high germination rate that increase the yield of the crop [11]. The mycoflora associated with test samples was studied in relation to the fungal succession, their effect on seed viability, biochemical changes in the seed, and fungal number every month. Seed-borne diseases have been found to affect the growth and productivity of crop plants [2]. Presence or absence of seed borne fungi on seed surface is one of the important aspects that determine the quality of seed [12].

A total of twenty fungal species belonging to twelve genera were isolated from the untreated seeds of variety Co13 (Table 1), the untreated seeds were found to be associated with highest number of seed borne fungi. About 15 species were isolated from treated seeds (0.1% Sodium hypochlorite), six from Bavastin, nine from Mancozeb, and nine from zeneb (z). *Aspergillus terreus, Mucor varians, Rhizopus nodosus* are the fungal species which had little effect with fungicide Bavastin. Growth of the fungal species *Cladosporium cladosporioides, Fusarium moniliforme, Penicillium griseofulvum, Rhizopus stolonifer, Trichoderma viride* could not be inhibited by Mancozeb. *Aspergillus nidulans, Curvularia* *lunata, Fusarium oxysporum, Fusarium solani, Penicillium griseofulvum, Pyrucularia setaria and Trichoderma viride were* insensitive against the fungicide Zeneb. Bavastin proved to be more effective than Mancozeb and Zeneb. It was reported that fungicides help to overcome the seed bore infection and help in control of diseases [13].

Table 1. Percentage frequency of fungal species in *Eleusine coracana* variety Co13

S. No	Fungal species	% frequency unsteralized	% frequency sterlized	% frequency Bavastin	% frequency Mancozeb	% frequency Zeneb
1	*Aspergillus flavus*	3.55	1.19	0.00	0.00	0.00
2	*Aspergillus nidulans*	4.79	1.19	0.00	1.19	0.90
3	*Aspergillus terreus*	10.16	5.69	3.19	0.00	0.00
4	*Curvularia lunata*	5.77	0.00	0.00	0.00	1.19
5	*Cladosporium oxysporum*	5.69	2.50	0.00	1.19	0.00
6	*Fusarium oxysporum*	2.50	1.19	0.00	0.00	1.19
7	*Fusarium moniliforme*	4.50	3.99	0.00	1.19	0.00
8	*Fusarium graminearum*	1.19	1.19	0.00	0.00	0.00
9	*Fusarium solani*	3.99	1.99	0.00	0.00	0.90
10	*Mucor varians*	14.87	6.24	1.99	0.00	0.00
11	*Penicillium griseofulvum*	3.69	0.00	0.00	1.19	0.09
12	*Phycomyces sp*	2.50	0.00	0.00	0.00	0.00
13	*Pyrucularia setaria*	2.50	1.19	0.00	0.00	0.09
14	*Rhizopus stolonifer*	9.94	3.71	0.00	1.19	0.00
15	*Rhizopus nodosus*	6.24	2.50	1.19	0.00	0.00
16	*Trichoderma viride*	5.33	3.71	0.00	1.19	1.19
17	*Yeasts*	5.33	1.19	0.00	0.00	0.00
18	*White sterile*	2.50	1.19	1.19	0.90	0.00
19	*Brown sterile*	3.71	1.19	1.19	0.90	1.19
20	*Black sterile*	1.19	1.19	1.19	0.90	0.09
	Total	99.94	41.04	9.94	9.84	6.83

The fungal species in variety Co 13 *Aspergillus flavus, Fusarium graminearum, Phycomyces sp* and *Yeasts* were totally eliminated by three fungicides Bavastin. Mancozeb and Zeneb. Bavastin and Mancozeb inhibited the growth of *Curvularia lunata, Fusarium oxysporum, Pyrucularia setaria*. Fungicide Mancozeb and Zeneb controlled the growth of *Aspergillus terreus, Mucor varians, Rhizopus nodosus. Cladospora cladosporioides, Fusarium moniliforme, Rhizopus stolonifer were eliminated by Bavastin and Zeneb.* Fungicide treatment induces metabolic changes and makes unfavorable conditions for the pathogens to grow Surface sterilization controlled the growth of *Curvularia lunata, Penicillium griseofulvum, Phycomyces sp* and *Yeast.* Similar [14] observation proved that surface disinfection of seed with 1% $Na(OCl)_2$ reduced the incidence of fungal pathogens.

A total of 18 fungal species belonging to seven genera were isolated from the untreated seed in the variety Co10 (Table-2). 13 from surface sterilized (0.1% Sodium hypochlorite), Eight fungal species *Fusarium graminearum, Penicillium funiculosum, Penicillium variable, Phycomyces sp, Yeasts* could not be controlled by fungicide Zeneb. Fungal patogens *Fusarium oxysporum, Phycomyces sp.* were eliminated by surface sterilization. It was observed fungicidal seed treatments are known to reduce the seed mycoflora and thereby *Alternaria alternate, Cladosporium oxysporum, Mucor sp, Penicillium funiculosum, Penicillium griseofulvum, Phycomyces sp, Rhizopus nodosus* and *Yeasts* were insensitive to Bavastin. Macozeb could not inhibit the growth of four fungi *Fusarium dimerum, Fusarium graminearum, Penicillium variable* and *Yeasts.* Sum of six species, *Fusarium moniliforme.*

Table 2. Percentage frequency of Fungal species in *Eleusine coracana* Variety Co10

S. No	Fungal species	% frequency unsterilized	% frequency sterilized	% frequency Bavastin	% frequency Mancozeb	% frequency Zeneb
1	*Alternaria alternata*	5.25	1.19	0.09	0.00	0.00
2	*Cladosporium oxysporum*	3.13	1.19	0.09	0.00	0.00
3	*Fusarium oxysporum*	1.19	0.00	0.00	0.00	0.00
4	*Fusarium dimerum*	5.25	3.69	0.00	0.90	0.00
5	*Fusarium moniliforme*	4.51	2.50	0.00	0.00	0.90
6	*Fusarium graminearum*	4.51	1.19	0.00	0.90	0.33
7	*Mucor sp*	7.64	2.50	1.19	0.00	0.00
8	*Penicillium funiculosum*	4.51	1.19	0.09	0.00	0.33
9	*Penicillium variable*	3.13	3.13	0.00	1.19	0.33
10	*Penicillium griseofulvum*	3.13	3.13	0.90	0.00	0.00
12	*Phycomyces sp*	4.51	0.00	0.90	0.00	1.99
13	*Pyruculari setaria*	4.51	3.13	1.19	0.00	0.00
14	*Rhizopus nodosus*	7.64	5.25	0.90	0.00	0.00
15	*Yeasts*	3.55	1.19	2.50	0.90	0.33
16	*White sterile*	14.77	6.24	1.90	1.90	1.90
17	*Brown sterile*	14.77	5.25	0.00	0.90	0.33
18	*Black sterile*	7.64	5.25	0.90	0.00	0.33
	Total	99.64	46.02	10.65	6.69	6.77

Fusarium graminearum, Penicillium funiculosum, Penicillium variable, Phycomyces sp, Yeasts could be controlled by fungicide Zeneb. Fungal patogens *Fusarium oxysporum, Phycomyces sp.* were eliminated by surface sterilization. It was observed fungicidal seed treatments are known to reduce the seed mycoflora and thereby improve the seed germination [15,16] Mancozeb was found effective in decreasing the associated seed fungi, followed by Zeneb and Bavastin. Presence of the dominant seed-borne fungi showed considerable mortality of seedlings. All the transmitted seed-borne fungi might well be a primary source of infection [17].

Fungal species *Alternaria alternate, Cladospora oxysporum, Mucor sp, Penicillium funiculosum, Penicillium griseofulvum, Phycomyces sp, Pyrucularia.setaria, Rhizopus nodosus, Yeasts* had little effect with Bavastin, fungicides Mancozeb and Zeneb completely inhibited them. Mancozeb could inhibit the growth of *Fusarium dimerum. Zeneb* decreased the associated seed fungi *Curvularia lunata, Fusarium moniliforme* but could not inhibit the growth. Fungal species *Fusarium oxysporum, Phycomyces sp* associated with the seeds were completely eliminated by surface sterilization. Germination of seed serves as an index of seed health. During the present study, untreated seeds result in poor germination and poor seedling vigor, resulting in an unhealthy crop. The fungi associated with seeds cause deterioration of seed quality, affect the viability and reduce germination. Control of seed-borne fungi will increase the percentage of seed germination and grain yield [18, 19] Seed-borne fungi reduces nutritional qualities of the seeds. The fungicides not only reduced the seed mycoflora improved seed germination. The low germination rate was due to the presence of microorganisms that affect the growth of newly emerging shoots. It was observed that germination occurred in all seeds with varying percentages. It means that there was an existing source of inoculums in the seeds, which will inhibit germination. The use of fungicides and sodium hypochlorite helped in minimizing the incidence of superficial and fast growing fungi as well as common seed borne fungi. Greater seedling emergence was obtained with fungicide-treated and seeds compared with fungicide-untreated Surface sterilized and control seeds.

Table 3. Germination percentage in Seeds of *Eleusine coracana*

Sr. No.	Variety		2nd month	4th month	6th month	8th month	10th month	12th month
1	Co13	unsterilized	60%	55%	50%	45%	40%	35%
		sterilized	80%	75%	65%	60%	55%	50%
		Bavastin	97%	95%	95%	95%	95%	95%
		Mancozeb	99%	95%	96%	96%	98%	98%
		Zeneb	98%	96%	98%	98%	98%	98%
2	Co10	unsterilized	70%	65%	60%	60%	55%	50%
		sterilized	85%	85%	80%	80%	80%	75%
		Bavastin	98%	98%	98%	97%	98%	98%
		Mancozeb	98%	97%	97%	98%	97%	98%
		Zeneb	97%	98%	97%	97%	97%	98%

References

1. Islam S.M.M., Masum M. M. I. and Fakir M. G. A. Prevalence of seed-borne fungi in sorghum of different locations of Bangladesh. Scientific Research and Essay 2009; 4(3):175-179.
2. Kubiak K. and Korbas M.: Occurrence of fungal diseases on selected winter wheat cultivars. Postepy Ochronie Roslin 1999; 39(2): 801-804.
3. International Seed Testing Association International rules for seed testing,: Proc. Int. Seed Asso 1966; 32:565-589.
4. De Tempe J.: The blotter method of seed health testing. Proc. ISTA 1953; 26: 133-151.
5. Neergard P.: Detection of seed borne pathogens by culture tests. Seed Sci. and Technol 1973; 1:217-254.
6. Agrawal, P. K.: Identification of suitable seed storage places in India on the basis of temperature and relative humidity condition. Seed Res 1976; 4(1):6-11.
7. Anon. International Seed Testing Association, International rules for seed testing: rules and annexes, Seed Science and Technology 1976; 4:1-177.
8. Raper, K. B. and Fennell O.J.: The genus Aspergillus. The Williams and Wilkins Co. Baltimore 1965; 686.
9. Booth C.: The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England 1971; 237.
10. Barnett, H.L. and Hunter B.B.: Illustrated genera of imperfect fungi (3Td) Ed; Burgess Publishing Company, Minnesota 1972; 24.
11. Zida, P.E., Sereme, P., Leth V,. Sankara P,. Somda I,. Néya A.: Importance of seed-borne fungi of sorghum and pearl millet in Burkina Faso and their control using plant extracts. Pak J Biol Sci 2008; 11(3):321-31.
12. Narayan, M,. Ghangaokar, and Ayodhya D Kshirsagar.: Study of seed borne fungi of different legumes, TLS. X 2009; 2(1):32-35.
13. Elizabeth Margaret., Neeraja P.V. and Rajeshwari, B. Sereenining of Seed Borne Mycoflora of Cicer arietinum L, Int. J. Curr. Microbiol. App. Sci 2003; 2(8)24-130.
14. Niaz, I. and Dawar, S.: Detection of seed borne mycoflora in maize (Zea mays L.) Pak J Bot 2009; 41:443–451.
15. Klich, M.A., Arthur, K.S., Lax, A.L. and Blade, J.M., Turin A. A potential new fungicide for stored grains. Mvcopathologia 1994; 127: 123-127.
16. Pande, A. and Varma, K.V.R.: Seed-borne fungi of pigeon pea, their pathogenicity and the fungicidal control. Biovigyanum 1992; 18:33-38.
17. Jonar I. Yago, Jae-Hwan Roh, Soon-do Bae, Young-Nam Yoon, Hyun-Ju Kim and Min-hee Nam.: The Effect of Seed-borne Mycoflora from Sorghum and Foxtail Millet Seeds on Germination and Disease Transmission Mycobiology 2011; Sep, 39(3):206–218.
18. DGISP.: Seed health. Food production, 1985: March.
19. Umechuruba, C.I. and N wachukwu, E.O.: Efficacy of certain fungicides against seed borne fungi of African yam bean seeds. I. J. Pest Manag 1994; 4 (2): 126-131.