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Comparative Efficacy of Different Incubation Methods for the Detection of Seed Borne Mycoflora of Mungbean(*Vigna radiata*L.)

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ABSTRACT
Seeds of three mungbeanvarieties viz., KKM-3, PS-16 and VBN-1 were examined for the detection of seed borne mycoflora by employing four incubation methods viz., standard blotter method, water agar method, deep freeze method and potato dextrose agar method. In all the three varieties standard blotter method was found more effective in enumerating the seed mycoflora of mungbean (12.9%) followed by potato dextrose agar method (11.2%). There isolated mungbean seed mycoflora were identified as Aspergillusniger, A. flavus, A. candidus, Alternariaalternata, Penicilliumnotatum, Rhizopustolonifer, Cladosporium sp., Fusariumoxysporum, Mucor sp., Curvularialunata, Chaetomiumglobosum and Macrophominaphaseolina.
Keywords: *Mungbean, Seed, Mycoflora,Varieties, Incubation, Methods etc.*

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INTRODUCTION

Mungbean [*Vignaradiata*L. Wilczek, syn. *Phaseolusaureus*Roxb, *P. radiates* L.] is a legume cultivated for its edible seeds and sprouts across Asia. The Mungbean seeds provide an invaluable source of digestible protein for humans in places where meat is lacking or where people are mostly vegetarian. In India, the name green gram is more commonly used than mungbean [1].

Seeds are the foundation to crop production and focal point in agriculture development without which an agriculture system is meaningless [2]. Seed health is an important factor in the control of diseases, since an infected seed is less viable, has low germination, reduced vigour and reduced yield [3]. The predominant mungbean seed mycoflora are *spergillusflavus, Aspergillusniger, Aspergillusterreus, Alternaria*sp*., Curvularia*sp*., Fusarium sp., Macrophominaphaseolina,* and*Penicillium sp.* [4]. Invasion by these mycoflora in storage might result in the discoloration of the seeds, rise in temperature, mustiness, loss in weight and various changes in the seed constituents. Some of the seeds infecting fungi produce mycotoxins such as aflatoxin, patulin, citrinine and ochratoxin [5].

Seed health testing by employing different incubation methods place an important role in detecting the seed borne mycoflora and helps in management and reducing losses caused by seed mycoflora. Keeping this in view, present investigation was envisaged.

MATERIALS AND METHODS

The seeds of commonly growing three mungbean genotypes *viz.*, KKM-3, PS-16 and VBN-1 were selected for the study. The seeds were sown in Petri plates either on moist blotter or on any other suitable synthetic or semisynthetic medium and were incubated for five to seven days. Four incubation methods were selected for this study *viz.,* standard blotter method, water agar method, deep freeze method and potato dextrose agar method. The details of each method is described below.

Standard blotter method

According to ISTA [6], four hundred seeds of each sample were placed equidistantly, aseptically on three layers of moist blotters moistened with sterile distilled water in sterile Petri plates of 90 mm diameter at the rate of twenty five seeds per plate and the plates were incubated for seven days under diurnal cycles of 12 h light and 12 h darkness at room temperature of 28±1 °C. After incubation seed mycoflora was recorded on eighth day by observing fungal growth on seeds under stereo binocular microscope. Further,

the species were confirmed by preparing slides and their frequency of occurrence was expressed in percentage. Statistical analysis was carried out by making use of arcsin transformation and two way ANOVA.

Water agar method

Four hundred seeds of each sample were placed equidistantly, aseptically on sterile Petri plates containing 1.5 per cent of water agar at the rate of twenty five seeds per plate. The plates containing seeds were incubated at the room temperature of 28±1 °C for seven days under diurnal cycles of 12 h light and 12 h darkness. Observations for seed mycoflora were recorded on eighth day after incubation by observing fungal growth on seeds under stereo binocular microscope and expressed in percentage. Statistical analysis was carried out by making use of arcsin transformation and two way ANOVA.

Deep freeze method

Deep freeze method is the modified blotter method followed for the experiment in the present study. In this method four hundred seeds were placed equidistantly, aseptically on three layers of moist blotters moistened with sterile distilled water in sterile Petri plates of 90 mm diameter at the rate of twenty five seeds per plate. The plates were incubated for 24 hours under usual conditions after which the plates were deep frozen for 24 hours under complete darkness. Observations for seed mycoflora were recorded after incubation by observing fungal growth on seeds under stereo binocular microscope and expressed in percentage. Statistical analysis was carried out by making use of arcsin transformation and two way ANOVA.

Potato dextrose agar method

Four hundred seeds of each sample were plated equidistantly, aseptically on sterile Petri plates containing potato dextrose agar at the rate of twenty five seeds per plate. The plates were incubated at room temperature of 28±1 °C for seven days under diurnal cycles of 12 h light and 12 h darkness. Observations for seed mycoflora were recorded on eighth day after incubation by observing fungal growth on seeds under stereo binocular microscope and expressed in percentage. Statistical analysis was carried out by making use of arcsin transformation and two way ANOVA.

RESULT

Four incubation methods *viz.*, standard blotter method, water agar method, deep freeze method and potato dextrose agar method were employed by using seed samples of three mungbean varieties *viz.*, KKM-3, PS-16 and VBN-1. The data was analysed for significance between the mycoflora, varieties and methods, the results of the study are presented in Table 1, Plate 1 and Figure 1.

Standard blotter method

Among four incubation methods, standard blotter method was found to be the best method for assessing the mycoflora. The maximum seed association of 25 per cent was rcorded by *F. oxysporum* in the variety KKM-3followed by *A. flavus*(21 %), *P. notatum* (20 %),*Cladosporium*sp.(18 %), *A. niger*(16 %), *M. phaseolina*(14 %), *R. stolonifer*(8 %),*Alternaria*sp.(8 %), *A. candidus*(6 %), *C. lunata*(4 %),*Mucor*sp.(3 %) and *C. globosum*(1 %).

Maximum seed association of 31 per cent by *F. oxysporum* was observed in the variety PS-16 followed by *A. niger*(25 %), *P. notatum* (24 %),*Cladosporium*sp.(23 %), *M. phaseolina*(19 %), *A. flavus*(16 %), *A. candidus*(8 %), *A. alternate* (7 %), *Mucor*sp.(6 %), *R. stolonifer*(5 %), *C. lunata*(5 %)and *C. globosum*(3 %).

In the variety VBN-1 Maximum seed association of 30 per cent was observed by *P. notatum*followed by *F. oxysporum*(26 %),*Cladosporium*sp.(22 %), *M. phaseolina*(18 %), *A. niger*(17 %), *R. stolonifer*(10 %), *A. candidus*(10 %), *A. flavus*(8 %),*Mucor*sp.(4 %), *A. alternate* (3 %) *C. lunata*(2 %),and *C. globosum*(1 %),

Water agar method

Among the three varieties studied under water agar method, maximum seed association of *M. phaseolina*(24 %) was observed in the variety KKM-3 followed by *P. notatum* (20 %), *Fusariumoxysporum*(15 %), *A. niger*(14 %), *A. flavus*(10 %), *R. stolonifer*(9 %),*Cladosporium*sp. (6 %) and *A. alternata*(1 %).

In the variety PS- 16, maximum seed association of *F. oxysporum*(21 %) was observed followed by *Penicillum*sp. (16 %),*Cladosporium*sp.(13 %), *A. niger*(8 %), *M. phaseolina*(8 %), *A. flavus*(6 %), *R. stolonifer*(2 %), *A. candidus*(2 %),%),*Mucor*sp.(1 %) and *C. globosum*(1 %)

Maximum seed association of *Cladosporium*sp. (21 %) was observed in the variety VBN-1 followed by *M. phaseolina*(20 %), *A. flavus*(17 %), *F. oxysporum*(15 %), *P. notatum* (13 %), *A. niger*(9 %), *A. candidus*(4 %), *A. alternate* (3 %) and *C. lunata* (2 %).

Deep freeze method

Among the three varieties studied under deep freezing method, maximum seed association of *F. oxysporum*(21 %) was observed in the variety KKM-3 followed by *P. notatum*(16 %),*Cladosporium*sp. (13

%), *A. niger*(8 %), *M. phaseolina*(8 %), *A. flavus*(6 %), *A. candidus*(2 %), *R. stolonifer*(2 %),*Mucor*sp.(1 %) and *C. globosum*(1 %)

The seed association of *F. oxysporum*(23 %) was maximum in the variety PS-16 followed by*P.notatum*(17 %), *A. niger*(10 %),*Cladosporium*sp. (7 %), *A. flavus*(5 %), *Mucor*sp.(2 %) *A. alternata*(1 %) and *C. lunata*(1 %).

Maximum seed association of *F. oxysporum*(16 %) was observed in the variety VBN-1 followed by *P. notatum*(12 %), *Cladosporium*sp. (11 %), *A. niger*(9 %), *M. phaseolina*(4 %), *A. flavus*(3 %), *R. stolonifer*(1 %),*Mucor*sp. (1 %) and *C. globosum*(1 %).

Potato dextrose agar method

Among the three varities studied under potato dextrose agar method, maximum seed association of *Penicillium*sp.(35 %) was observed in the variety KKM-3 followed by *Fusariumoxysporum*(34 %),%), *M. phaseolina*(25 %), *A. niger*(20 %), *A. flavus*(12 %),*Cladosporium*sp.(12 %), *A. alternata*(4 %) and*Mucor*sp.(3 %).

In the variety PS-16 maximum seed association was observed by of*F. oxysporum*(56 %) followed by *P. notatum*(45 %), *A. flavus*(14 %), *Cladosporium*sp.(10 %), *A. niger*(8 %), *M phaseolina*(4 %), *C. lunata*(3 %) and *A. candidus*(2 %).

Maximum seed association of *F oxysporum*(36 %)observed in the variety VBN-1 followed by *P. notatum*(31 %), *Cladosporium*sp. (20 %), *A. niger*(12 %), *A. flavus*(9 %), *A. candidus*(3 %), *A. alternata*(3 %) , *C. lunata*(1 %) *Mucor*sp.(1 %) and*R. stolonifer*(1 %).

DISCUSSION

Standard blotter method was significantly different in comparison with other methods and was found best in enumerating the seed mycoflora (12.9%) in all the three varieties fallowed by potato dextrose agar method (11.2%) water agar method(7.8%) and deep freeze method (5.6%). Several workers have been reported that blotter method as the best method for identification of seed mycoflora in different crops [7,8,9&10]. As reported by earlier workers, during the present investigation also standard blotter method was found the best method for the assessment of seed mycoflora.

Table 1: Seed mycoflora of mungbean varieties under different incubation methods

Incubation methods	Varities	Seed germination (%)	Per cent seed mycoflora													
			Aspergillus niger	*Alternaria alternata*	*Curvularia lunata*	*Aspergillus flavus*	*Aspergillus Candidus*	*Cladosporium* spp.	*Fusarium oxysporum*	*Penicillium notatum*	*Macrophomina Phaseolina*	*Rhizopus stolonifer*	*Mucor*spp.	*Chaetomium globosum*	Mean seed mycoflora (%)	Mean seed mycoflora Of the varities
Standard blotter method	KKM-3	88	16	8	4	21	6	18	25	20	14	8	3	1	12.0 (20.27)*	12.9
	PS-16	91	25	7	5	16	8	23	31	24	19	5	6	3	14.3 (20.22)	
	VBN- 1	89	17	3	2	8	10	22	26	30	18	10	4	1	12.6 (20.79)	
Water agar method	KKM-3	86	14	1	0	10	0	6	15	20	24	9	0	0	8.3 (16.74)	7.8
	PS-16	90	8	0	0	6	2	13	21	16	8	2	1	1	6.5 (14.77)	
	VBN- 1	87	9	3	2	17	4	21	15	13	20	0	0	0	8.7 (17.15)	
Deep freeze method	KKM-3	85	8	0	0	6	2	13	21	16	8	2	1	1	6.5 (14.77)	5.6
	PS-16	77	10	1	1	5	0	7	23	17	0	0	2	0	5.5 (13.56)	
	VBN- 1	79	9	0	0	3	0	11	16	12	4	1	1	1	4.8 (12.66)	
Potato dextrose agar method	KKM-3	86	20	4	0	12	0	12	34	35	25	0	3	0	12.0 (20.27)	11.2
	PS-16	88	8	0	3	14	2	10	56	45	4	0	0	0	11.8 (20.09)	
	VBN- 1	83	12	3	1	9	3	20	36	31	0	1	1	0	9.8 (18.24)	

Mean	13.0 (21.13)	2.5 (9.10)	1.5 (7.03)	10.6 (19.0)	3.1 (10.14)	14.7 (22.54)	26.6 (31.05)	23.3 (28.86)	12.0 (20.27)	3.2 (10.30)	1.8 (7.71)	0.7 (4.80)	-
	Mycoflora		**Varieties**		**Incubation method**								
SEm±	2.40		1.43		1.38								
CD (0.01)	8.49		4.98		4.77								

*The values in the parenthesis are arcsin transformed values

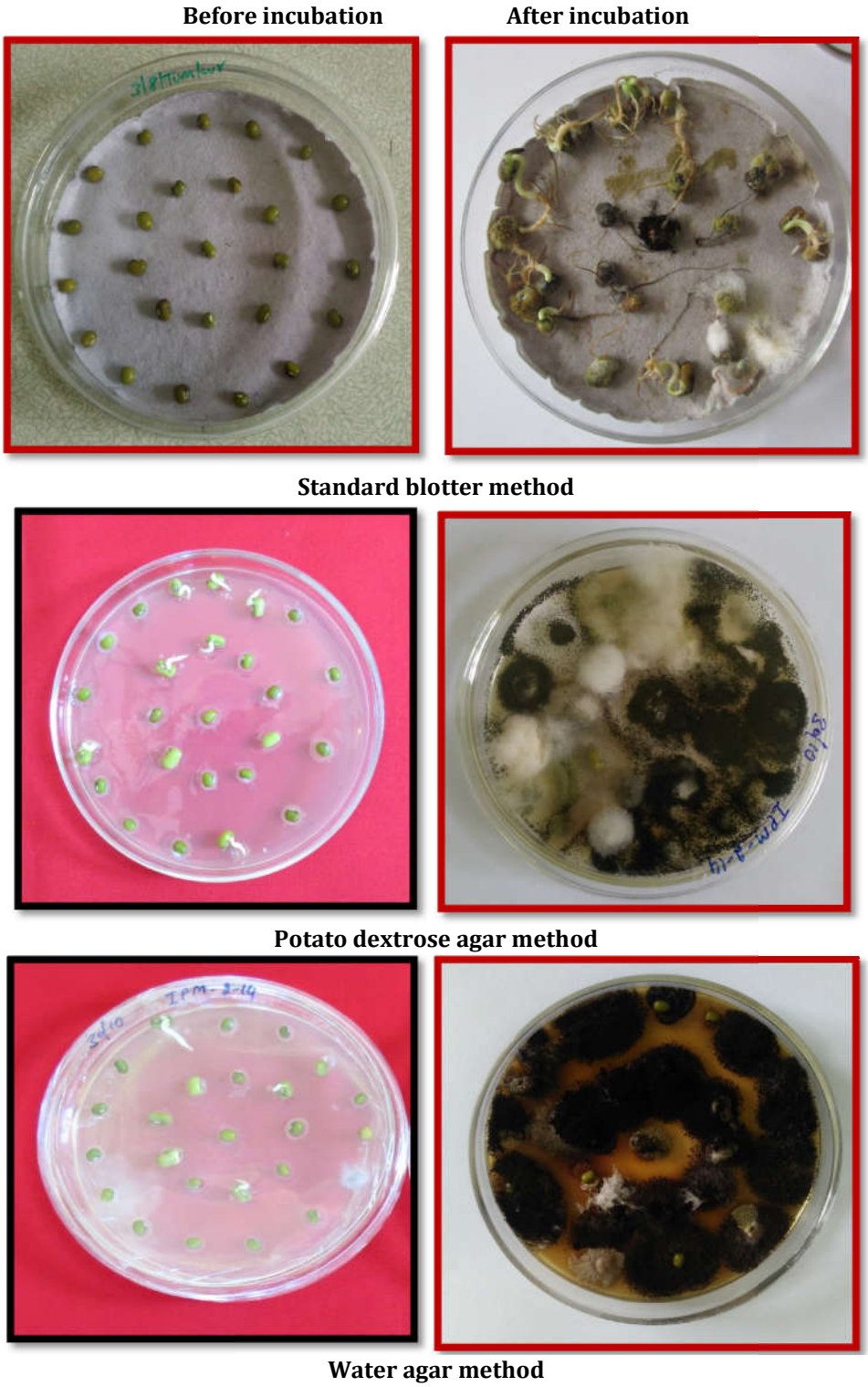

Plate 1: Different incubationmethods

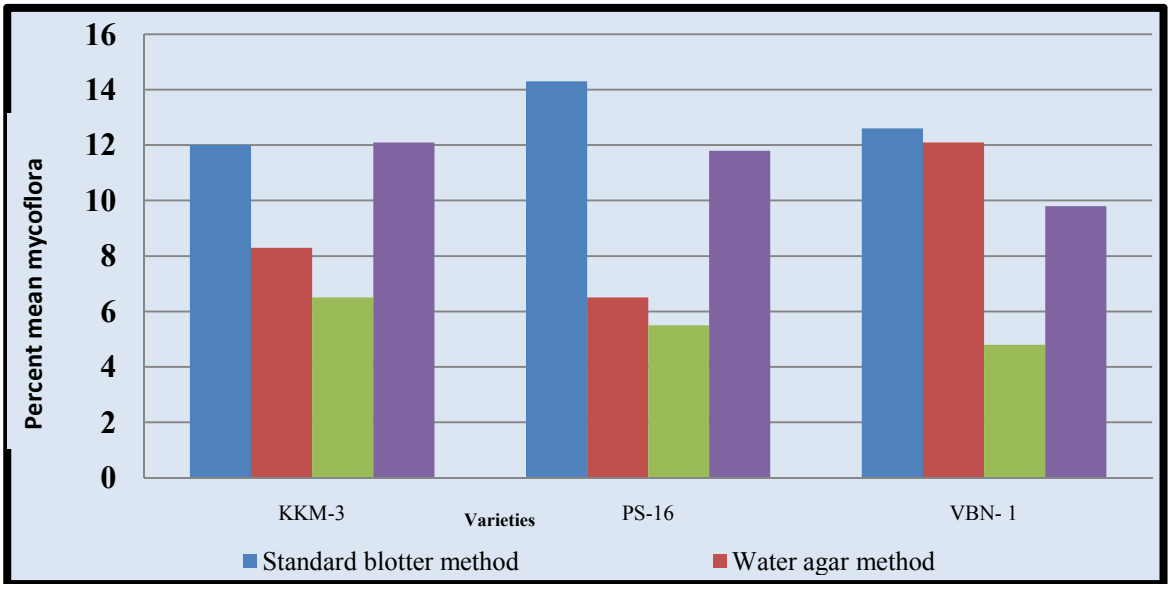

Fig. 2: Seed mycoflora on mung bean varieties in different incubation methods

CONCLUSION

Out of four incubation methods tested standart blotter method was found best in enumerating the seed mycoflora (12.9%) in all the three varieties of mungbean fallowed by potato dextrose agar method (11.2%) water agar method(7.8%) and minimum expression was found in deep freezing method(5.6%).

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