HELIA, **15**, Nr. 17, p.p. 73-80 (1992)

COMPARATIVE STUDIES ON SUNFLOWER SEED HEALTH TESTING METHODS AND THEIR ECONOMICS

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SUMMARY

Of the four different methods used for comparative studies, the dry inspection method was found to be very cheap and not time consuming but its sensitivity was very poor in testing sunflower seed for health status. The agar plate method was found very expensive and time consuming with low sensitivity in routine testing. The anchor paper method yielded high number of fungi and was found more time saving and economic as compared with the agar method.

Seed recovery percentage was very high on the seven hybrid/variety of sunflower tested by the blotter paper method and found more simple, time saving and economic in routine seed health testing of sunflower than the other three methods.

Key word: Seed health testing, agar plate method (PDA), sunflower seed.

INTRODUCTION

Sunflower (*Helianthus annus, L*) is a major non–conventional oil seed crop, planted at over 78000 hectares in Pakistan (Anonymous, 1991). The big gap between edible oil production and consumption has increased the importance of sunflower crop which has 40–42 percent oil content (Beg, 1983). With an increase in the acreage, new pathological problems will also emerge. More than a dozen diseases including charcoal rot (*Macrophomina phaseolina*), Alternaria leaf spots (*Alternaria* spp.) Septoria leaf spot (*Septoria helianthi*), head rot (*Rhizopus* spp.) rust, (*Puccina helianthi*), powdery mildew (*Erysiphe cochoracearum*), stalk rot (*Sclerotia sclerotiorum*), and collar rot (*Sclerotiorum rolfsii*) have been reported from Pakistan (Mirza and Beg, 1983., and Burney et al., 1988). Most disease causing microorganism in sunflower are reported to be seed–borne in nature (Richardson, 1990). In Pakistan, Dawar and Ghaffar (1991) isolated 36 species of fungi from 20 sunflower seed samples by different methods, but their sensitivity with regard to simplicity, time and economics is not known.

Detection of seed–borne pathogens is an important factor in the production of quality seed to ensure healthy and vigorous crop. In this regard, seed health testing methodology needs to be suitable, quick and economic. This will help not only in testing large

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quantities of seed samples under seed health certification system but also increase efficiency in screening huge seed materials in research. In this investigations a comparative study on different methods used for detection of various fungi, i.e. agar plate method, blotter paper method, anchor paper and dry inspection technique was carried out on sunflower seed samples collected from Punjab, Sindh and NWFP in Pakistan.

MATERIALS AND METHODS

Seed sample of seven sunflower cultivars, SF–100 Turbish, SMH–138, C–206, Suncome–90, Ho–1, and NK–212, drawn from imported seed stuff, were used in this study during 1991. Samples were colected by the procedure as laid down in ISTA rules (Anonymous, 1985). No chemical treatment was given to any seed sample.

DETECTION OF FUNGI

1. *Blotter Paper Method*: Four hundred seeds for each sample were tested using the standard blotter paper method (Anonymous, 1985). Twenty seeds were placed on three layers of moistened blotters (AGE, Tekniske papirer –Dk) in 9–cm glass petri dish (Pyrex). Seeds were incubated for eight day at 20°C (+2) under alterating cycles of 12 hours day and night. Two sets of four hundred seed were plated from each sample for the mean average of time required in testing. Seeds were examined under stereo– microscope (Wild Heerburgg with two light sources magnification 6x50). Fungi were identified based on habit characters. Confirmation of species was done with the help of compound microscope (Leitz Laboralux –D, magnification 100x400) where necessary (Barnett, 1960; Nelson et al., 1983).

2. *Agar Plate Method*: In the agar plate method, potato dextrose agar (PDA) medium was used. Medium ingredients were weighed by Ohaus Brain weigh electronic balance (Ohaus model B – 5000). Thirty nine grams of PDA (Difco produce) were autoclaved in 1000 ml of distilled water at 15 lbs/sq inch pressure for 20 minutes (locally manufactured autoclave). About 20 ml of this sterilized medium was poured in each petri dish (9 cm diameter, Pyrex, USA) in laminar flow (Gelair SN 82501, Italy) to avoid any contamination. Ten seeds were plated in each petri dish. Recording procedure was similar as described earlier. Time required for each step, i.e., weighing, autoclaving, pouring, and plating, were also recorded:

3. *Anchor Paper Method*: In this experiment, anchor brand paper (often used for germination test) was used instead of blotter paper. The sheets of anchor brand paper was cut to the size of blotter paper. The rest of the procedure was similar to the blotter paper method. Time required for each step, i.e., cutting the anchor brand paper, plating and observation, were noted.

4. *Dry Inspection Method*: Hundred grams of sunflower seeds were weighed by Ohaus electronics balance and put on anchor brand paper. Seeds were directly examined under the stereomicroscope (Wild Heerburgg) for sclerotial and pycnidial contaminants and results were prepared.

Cost of substrate and other materials, i.e., blotter paper, PDA, anchor brand paper and aluminum foil paper was calculated for each sunflower seed health testing method where these were used.

Table 1 – Comparative study on sunflower seed health testing methodology

		Testing methods (types of substrate) and percentage recovery of seed–borne fungi							
Acc. No.	Hybrid/ Variety	Dry inspection	Blotter Paper		Anchor brand paper		Agar plating		Remarks
1.	2.	3.	4.		5.		6.		7.
1	SF–100	Sclerotia of scler. sp. Nil	Nil		*Alternaria tenuis*	0.5%	Nil		Heavy growth of Rhizopus sp.
24	Turbish	Nil	*Alternaria tenuis*	63.5%	*A. tenuis*	63.5%	*A. tenuis*	4.0%	Aspeegillus sp.
			Cephalosporium sp.	1.0%	*Cephalosporium* sp	6.0%			Mucor
			Fusarium semitectum	2.0%	*F. moniliforme*	1.5%			Trichoderma sp.
			F. Solani	1.0%	*F. semitectum*	1.5%			
31	SMH–138	Nil	*A. Tenuis*	91.0%	*A. tenuis*	17.0%	*A. tenuis*	11.0%	Less seporphytes.
			Cephalosporium sp.	1.0%			*Cephalosporium* sp.	2.0%	
			D. hawaiiensis	1.0%			*F. moniliforme*	1.0%	
			F. moniliforme	0.5%			*F. semitectum*	3.0%	
58	C–206	Nil	*A. tenuis*	0.5%	Nil		Nil		Heavy growth of Rhizopus sp.
77	Suncome-90	Nil	*A. tenuis*	57.0%	*A. tenuis*	27.0%	*A. tenuis*	4.0%	Less seprophtes
			Phoma sp.	0.5%	*F. semitectum*	0.5%	*F. moniliforme*	1.0%	
			Stemphyllium	1.0%			*F. semitectum*	1.5%	
96	HO–1	Nil	*A. tenuis*	72.5%	*A. tenuis*	59.0%	*A. tenuis*	28.0%	– do –
			Cephalosporium sp.	3.5%	*Cephalosporium* sp.	1.0%	*F. moniliforme*	2.5%	
			F. moniliforme	2.0%	*F. moniliforme*	1.0%	*F. semitectum*	4.0%	
			F. semitectum	1.5%	*F. semitectum*	2.0%			
146	NK–212	Nil	*A. tenuis*	57.0%	*A. tenuis*	30.0%	*A. tenuis*	10.0%	– do –
			D. tetramera	1.0%	*M. Phaseolina*	1.0%	*D. terramera*	0.5%	
			Macrophomina		*Phoma* sp.	0.5%	*M. Phaseolina*	3.0%	
			Phaseolina	4.0%			*Phoma* sp.	1.0%	
			Phoma sp.	2.0%					

RESULT AND DISCUSSION

Fungi on seven sunflower hybrid/ variety seed samples, recorded by using four seed health testing methods are, presented in Table 1.

COMPARISON OF INCIDENCE OF FUNGI

The percentage of recovery of various fungi was different in the four methods. A large number of fungi with high seed recovery percentage was recorded in blotter paper in all the seven sample tested. High incidence of *Alternaria tenuis* was 91.0 percent on the cultivar SMH–138 while in the anchor brand paper method it was 59.0 and in the agar method 28.0 percent on HO–1.

Obviously, it was due to a lower water holding capacity of anchor brand paper and quick growth of saprophytes in the case of the agar plate method. It was observed that it is difficult to assess the exact amount of moisture in testing period for the anchor paper method. So in providing additional moisture, the condition inside the petri dish may be disturbed as a proper and regular growth of fungal habitat. These critical factors may have affected the fungal recovery. In the case of the agar method (PDA) for cotton, Bhutta (1988) observed that seed health testing is very difficult in the presence of fast growing saprophytes and sclerotial fungi. Sclerotial fungi on one seed may quickly spread on other seeds and it may cause confusion in counting recovery percentage. Khan et al. (1988) and Dawar & Ghaffar (1991) observed similar results in detecting seed–borne mycoflora of rice and sunflower by the blotter paper and the agar plate method, respectively. The agar plate method was used for large scale health testing of flax seed lots in Northern Ireland for 15 years by Muskett and Malone (1941). A modified special agar plate method (water agar) was used by Mangan (1971) for detection of *Phoma betae*. The agar plate method is applicable to those seeds in which saprophytic species do not materially impair quick identification of the pathogens, a difficulty overcome by pretreatment. In case of sunflower seed, the involvement of saprophytic fungi in the production of mycotoxin is also important (Parasad and Singh, 1983). In routine seed health testing for seed– borne fungi, pathogens are also checked along with saprophytic fungi (Neergaard, 1979).

The dry inspection technique is not suitable for sunflower seed health testing as no organism could be detected including any sclerotial or pycnidial contaminants (Table 1).

Comparison of the methods for time and cost

Although the blotter paper and agar plate method have been reported to be simple and suitable for routine seed health testing for a number of pathogens in seeds of different crops seed (Neergaard, 1979), these are not in routine use in Pakistan. One of the reasons is that the suitability with respect to time and cost is not appropriate for Pakistan. The same incubation conditions were given to each sample except in the anchor brand paper method where additional moisture was given during incubation period. Total time required and cost incurred for each method is were given in Tables 2 and 3. Eight days of incubation period given to each method except the dry inspection method which does not require any incubation. It is evident from Tables 2&3 that the agar plate method much time (200 minutes) was consumed and higher cost (Rs.65.0) was requiredas compared the blotter paper, anchor paper and dry inspection methods. Cost (Rs.2.0) and

time (95 minutes) per sample in the case of the dry inspection technique was found to be lower than for the other methods under study but suitability of this method was found very poor with respect to seed recovery of the mycoflora of sunflower.

In this study, the blotter paper method was found suitable, simple, less time consuming and more economical than the agar plate and anchor paper method. It took 115 minutes to complete full results by incurring Rs 10.00 per sample. Since seed health is an important component of quality seed in controlling seed–borne diseases, every seed lot should be tested for health status. In Pakistan, 4000 sunflower seed samples are expected to be tested each year. If it is tested by the agar plate method, an amount of Pak. Rs. 260,000 is needed while in the case of the blotter paper method, the cost is only

Table 2 – Time required for detection of fungi by diferent methods per seed sample of sunflower

Sr. No.	Recording factors	Time consumed to complete each method (Minutes)				
		Dry inspection method	Bloter paper method	Agar plate method	Anchor paper method	Remarks
1.	Plating of seed.	Nil	35	45	40	1). Additional moisture was given in anchor brand paper method only. 2). Other factors kept constant e.g. 8 days incubation period except for dry inspection method for which no incubation is required. * only weighing
2.	Weighing & mixing of ingredient.	*5	Nil	10	Nil	
3.	Autoclaving	Nil	Nil	60	Nil	
4.	Pouring of medium.	Nil	Nil	20	Nil	
5.	Cutting for paper.	Nil	Nil	Nil	20	
6.	Observation/ recording	85	65	55	60	
7.	Preparation of result	5	15	10	15	
	Total:	95	115	200	135	
Note: Mean average of observation/recording time for the set of experiment.						

Table 3 – Expenditure involved on substrates used in detection of fungi by different methods per seed sample of sunflower (january, 1992)

S.No	* Substrate	Cost incurred per sample (Pak.Rupees)			
		Dry inspection method	Blotter paper method	Agar plate method	Anchor paper method
1.	Blotter paper	Nil	10	Nil	Nil
2.	Potato dextrose agar (PDA,DIFCO)	Nil	Nil	64.00	Nil
3.	Anchor Brand paper	2.00	Nil	Nil	16.00
4.	Aluminium foil paper	Nil	Nil	1.00	Nil
	Total:	2.00	10.00	65.00	16.00

*: Cost of glassware, equipment, supporting items and electricity charges kept constant.

Rs.40,000. The blotter paper method could be reliable and economical in screening large scale seed samples by researsch workers. In this respect, millions of rupees can be saved. This method could be used with larger reliability of results than the agar plate method for sunflower seed health testing against fungi in poorly equipped laboratories in Pakistan.

ACKNOWLEDGMENT

The senior author is thankful to Syed Irfan Ahmad, Director General, Federal Seed Certification Department, Islamabad, for granting permission to publish this paper.

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ESTUDIOS COMPARATIVOS DE METODOS DE SANEAMIENTO DE SEMILLA DE GIRASOL Y SU COSTE ECONOMICO

RESUMEN

De los cuatro méthodos diferentes utilizados para estudios comparativos, un método de inspección en seco fue encontrado muy barato y mas rápido pero su sensibilidad fue muy pobre para testar el estado sanitario de las samillas de girasol. El método de la placa de agar se enoontró caro y largo con poca sensibilidad en testado de rutina. El método de papel de ancla detectó un número de hongos alto y fue ahorro mas tiempo y fue mas económico comparado con el método de agar.

El porcentaje de recuperación de semillas fue muy alto en las siete variedades/híbridos de girasol testadas por el método del papel secante y fue encontrado mas simple, rápido y económico en testado de sanidad de semillas que los otros tres métodos.

ETUDES COMPARATIVES SUR L'ÉTAT SANITAIRE DES GRAINES DE TOURNESOL. MÉTHODES ET CONSIDÉRATIONS ÉCONOMIQUES

RÉSUMÉ

Sur quatre différentes méthodes utilisées pour des études comparatives, la méthode d'inspection à sec a été trouvée très peu couteuse et peu consommatrice de temps mais la précision a été préuvue pour tester l'état sanitaire des graines de tournesol. La culture sur agar a été trouvée très chère et consommatrice de temps avec une faible sensibilité pour du test de routine. La méthode "anchor paper" a produit un grand nombre de champignons el a été trouvée plus économique en temps et en coût que la méthode agar.

Le pourcentage de reprise des graines a été très élévé sur sept hybrides/variétés de tournesol testées par la méthode du papier buvard, qui a été trouvée très simple, économique en temps et en coût comme methode de routine pour caracteriser l'état sanitaire des graines en comparaison avec les trois autres méthodes.