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Assessment of various seed-borne fungi association in onion (*Allium cepa L.*) seeds by blotter paper and PDA method of isolation and detection

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ABSTRACT

Onion is one of the most important commercial vegetable crops grown in Pakistan. It is valued for its distinct pungent flavor and is an essential ingredient for the cooking in many regions. Seed born fungus on onion seed cause seed.abortion, seed.rot, sclerotisation or stromatisation of seed, seed.necrosis, decrease or removal of germ inability and sprout injury which resulting in the growth of ailment in later-stages of plant development by universal or native poison. In this study, seed born fungus was detected from infected onion seeds by using blotter paper method and agar plate method. Seed born fungus

was detected by using different fungus detecting methods, consequence of seed born fungus on seed sprouting. The results obtained that the maximum incidence of *A. porri* (4%), *A. niger* (2.29%) and *A. flavus* (1.50%) while minimum incidence of *F. oxysporum* (0.99%) and *Botrytis* spp. (0.67%) were recorded.

Key words: Fungus, Blotter, PDA, Incidence

INTRODUCTION

Onion scientifically known as *Allium cepa* L. is important bulb crop commercially cultivated throughout the world, belongs to genus Allium and is member of family Alliaceae (Hanelt, 1990). It is cultivated for the consumption both in mature bulbs and in the green state as well. Onions show specific diversity in Eastern Mediterranean countries, through Tajikistan, Turkmenistan to India and Pakistan that are believed to be the center of origin and very significant sources of genetic diversity (Brewster, 2008).

Onion is vital profit-making vegetable crop cultivated in Pakistan and it is thought to be native to Mediterranean and south west Asia (Wani and Nisa, 2011). Total area under onion cultivation is 1.64 million hectares of the world while total onion yield is 86.34 million tones (FAO, 2011). Area in Pakistan under onion cultivation is about 143.7 thousand hectares and production is about 1892 thousand tons in the year 2010-11 (FAO, 2011). Onion (*Allium cepa* L.) is cultivated for mild or pungent flavours, it form necessary ingredient in many people diet. Onion distinctive characteristic is because of the presence of alliaceous odour that account for its use as salad, food, condiment, spice and in medicine (Raju and Naik, 2007). Norman (1992), reported that onion has relatively high food value, rich in riboflavin and calcium, intermediate in protein. It is the plant of dry, sunny, open sites in the dry climates, whereas various species are found also in arid mountain slopes, steppes, stony or rocky open sites, or dry summer (Hanelt, 1990).

Quality and yield of dry bulbs might be influenced by growing environment and cultural practices. Hence, research in country was focused mainly on identification of superior onion cultivars and adopting upgraded management practices for healthier yield, and cultural practices like plant to plant spacing to control yield, shape and bulb size (Geremew *et al.,* 2010). Generally, the post-harvest diseases increase during rainy season perhaps due to high relative humidity. Raju and Naik (2007), observed that it is because of high relative humidity and favourable temperature during wet season. Aveling *et al.,* (1993) isolated similar fungi mentioned above from infected onion seeds in South Africa. Among tested onion seeds *A. niger* was most encountered fungi. It verifies the results of El-Nagerabi and Abdalla (2004) that commonly encountered genus from onion seeds examined in Sudan was Aspergillus. *A. niger* high prevalence on onion seeds could attribute to its ability to use the flowers vulnerability to pierce the seeds (Sirois and Lorbeer, 1998). It also causes reduction in seedling emergence, seed germination and vigour (El-Nagerabi and Ahmed, 2001; Koycu and Ozer, 1997).

Hayden *et al.,* (1994) reported that *A. niger* can also be transmit to stored onion bulbs from contaminated seeds to cause the disease Black mould. Fungal pathogens are mostly controlled by chemical compounds (Wainwright, 1979; Mathur and Sharma, 2006; Mathur *et al.,* 2007). These synthesized pesticides cause contamination and disturb the micro biotic balance in soil. In natural conditions, microbes interact with each other with competition and hyper parasitism, checking the growth and reproduction of several other harmful plant pathogens and maintain ecological balance.

The fungal pathogens associated with damping off are *Pythium spp., Rhizoctonia solani* and *Fusarium oxysporium*. Lifshitz *et al.,* (1986) found that production of a toxic factor by *Trichoderma harzianum* and *T. koningii* inhibits growth of *Pythium spp*. The antibiotics, gliovirin and gliotoxin isolated from *Trichoderma virens* described as strong inhibitory compound to *Pythium spp.* and *Rhizoctonia solani* (Weindling, 1934; Howell and Stipanovic, 1983). Loss of gliotoxin production due to induced mutation in *T. virens* had an adverse effect on the efficacy of mutant strains as bio-control agents of Pythium damping-off (Wilhite *et al.,* 1994).

Elevated populations of Pseudomonas spp., lower pH values, high organic matter, K, Mg and Ca contents in soil showed suppression of damping off disease by reducing the rate of oospores germination in the soil (Lumsden *et al.,* 1987). Neem oil extract was found effective to reduce colony diameter, dry weight and soprulation of *Fusarium oxysporum*. The extract inhibited the production of fusaric acid, involved in the disease development (Govindachari *et al.,* 1998). Application of bio-agents, *Bacillus subtilis, Pseudomonas fluorescens* and *Trichoderma harzianum* exhibited highest reduction in disease severity (Hussein *et al.,* 2007).The research was carried out to record and observe onion seed mycoflora, stored under different conditions and to check various seed treatment fungicides effectiveness.

MATERIALS AND METHODS

Appearently healthy seeds samples of five onion varieties Phulkara, Desi red, Early red, Robina and Dark Red were collected from grain market Faisalabad. Collected healthy samples were subjected to initial determination of seed-borne fungi by Agar plate and Standard Blotter Method (Quenton *et al.,* 2003). Different fungi were isolated, identified and purified, via fungal growth pattern, colony color and sporulation type and with the help of literature (Sutton, 1980, Booth, 1971). Fungi were identified on the basis of spore morphology and colony characters (Barnett and Hunter, 1972) and Dematacеous hyphomycetes (Ellis, 1971). Fungi were isolated and culture was identified with the help of available literature. Single spore technique was used for purification of isolated fungi (Tuite, 1969).

PDA METHOD WITH POTATO DEXTROSE AGAR

PDA method was used for seed. A 500 onion seeds from all cultivars were surface sterilized with one percent sodium hypochlorite for 3-4 minutes and then washed 2-3 times with sterile water. 10 seeds

were placed in petri-plate with 20 ml PDA media. Plates were incubated at (25±2ºC) for 7-9 days in the incubator. After seven days of incubation associated fungi were observed under microscope.

STANDARD BLOTTER METHOD

This method was developed in 1938 by Doyer, included in ISTAR rules 2016. In this method 400 seeds of every variety were tested with 3 replications. Petri-plates used having 90 mm size were used. Two pieces of blotter paper 90 mm size first soaked in distilled water and then placed in petri plates, after draining excess water. 10 seeds per plate were placed on blotter paper. Then inoculated petri plates were placed at 25±1°C under alternate cycles of twelve hours NUV light and darkness. Seeds were examined after 5-7 days under stereoscopic-binocular microscope for associated seed borne fungi (Anon., 1996).

Four different seed dressing fungicides were used to check the effect of fungicide for control of seed-borne mycoflora. Each variety was subjected to seed dressing fungicides. Blotter paper method was used for testing these fungicides under in vitro conditions, (Nene and Thapliyal, 1973). The experiment was carried out under complete randomized design (CRD) in vitro and randomized complete block design (RCBD) in vivo, statistical analysis was performed using standard analysis of variance (ANOVA). Standard error of means (SEM) and Means were calculated and statistical significance between the mean values was assessed by using Least Significant Difference (Steel *et al.,* 1997).

RESULTS

During the evaluation of detection methods the fungus incidence was observed at higher level in PDA method (15.94%) as compared to Blotter paper method (13.47%) Table 1 shows that maximum fungal incidence was observed in case of PDA method as compared to blotter paper method. *Alternaria porri* showed maximum incidence while *Botrytis* spp. exhibited minimum incidence.

Table 1: Analysis of Variance for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Phulkara.

Source	DF	SS	MS	F	P
Fungus	4	1554.89	388.722	319497	0.0000
Method	1	45.78	45.781	37628.6	0.0000
Fungus×Method	4	3.71	0.927	761.53	0.0000
Error	20	0.02	0.001		
Total	29	1604.40			

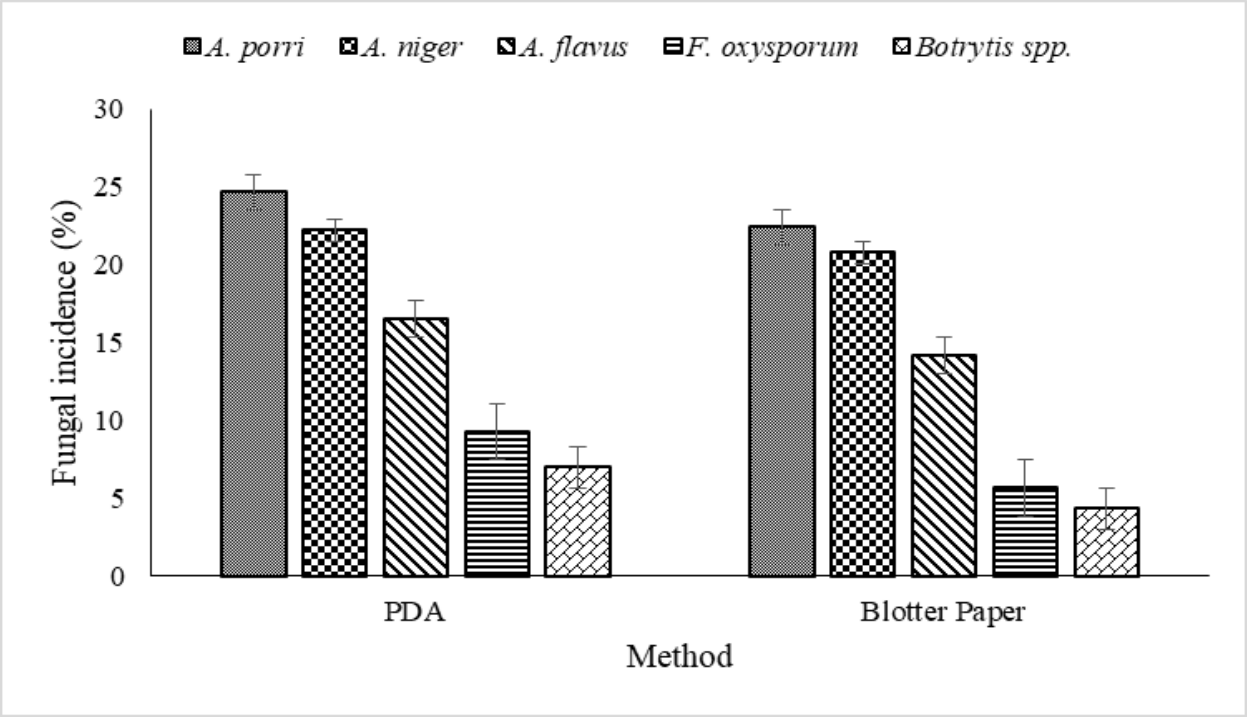

Fig. 1. Graphical representation of mean values of for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Phulkara.

During the evaluation of detection method the fungus incidence was observed at higher level in PDA method (14.34%) as compared to Blotter paper method (11.87%) Table 2 shows that maximum fungal incidence was observed in case of PDA method as compared to blotter paper method. *Alternaria porri* showed maximum incidence while *Botrytis* spp. exhibited minimum incidence.

Table 2: Analysis of Variance for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Desi Red.

Source	DF	SS	MS	F	P
Fungus	4	1366.59	341.647	280806	0.0000
Method	1	45.78	45.781	37628.6	0.0000
Fungus × Method	4	3.71	0.927	761.53	0.0000
Error	20	0.02	0.001		
Total	29	1416.10			

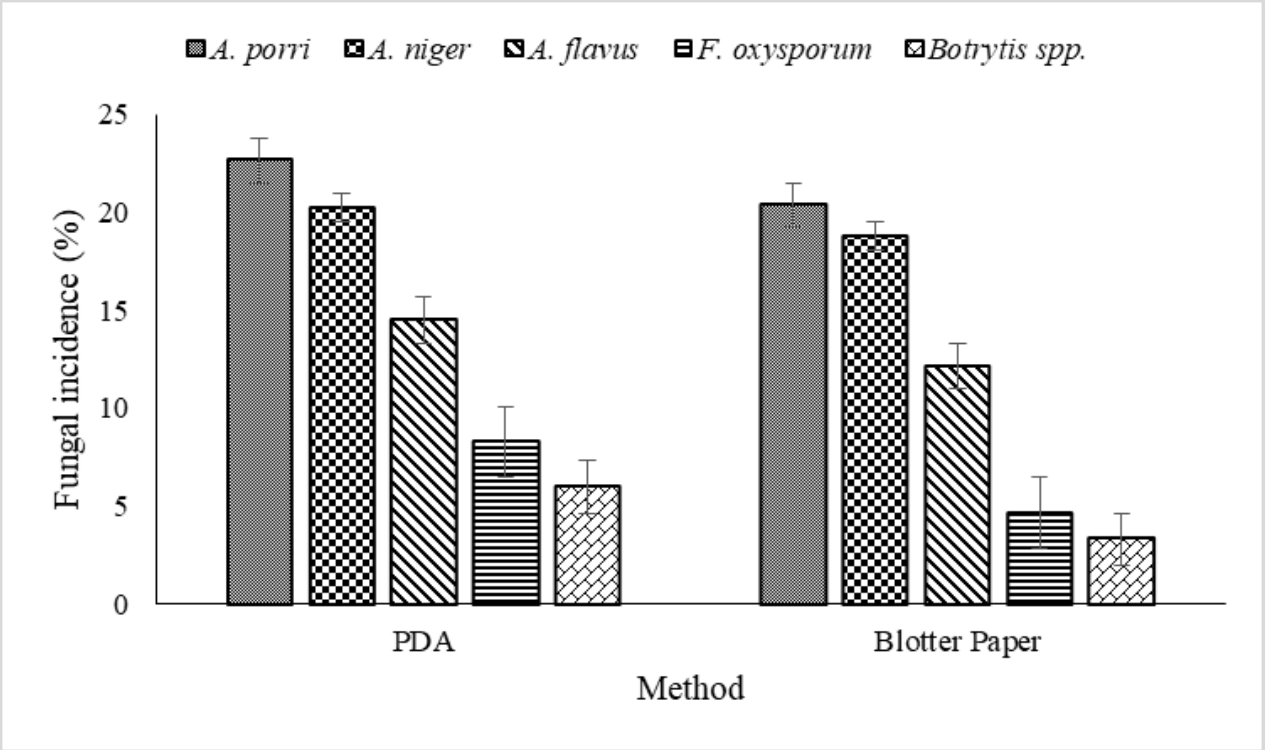

Fig.2. Graphical representation of mean values of for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Desi Red.

During the evaluation of detection methods the fungus incidence was observed at higher level in PDA method (12.21%) as compared to Blotter paper method (9.47%) Table 3 shows that maximum fungal incidence was observed in case of PDA method as compared to blotter paper method. *Alternaria porri* showed maximum incidence while *Botrytis* spp. exhibited minimum incidence

Table 3: Analysis of Variance for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Early Red.

Source	DF	SS	MS	F	P
Fungus	4	840.071	210.018	5355.32	0.0000
Method	1	56.197	56.197	1433.00	0.0000
Fungus × Method	4	8.493	2.123	54.14	0.0000
Error	20	0.784	0.039		
Total	29	905.546			

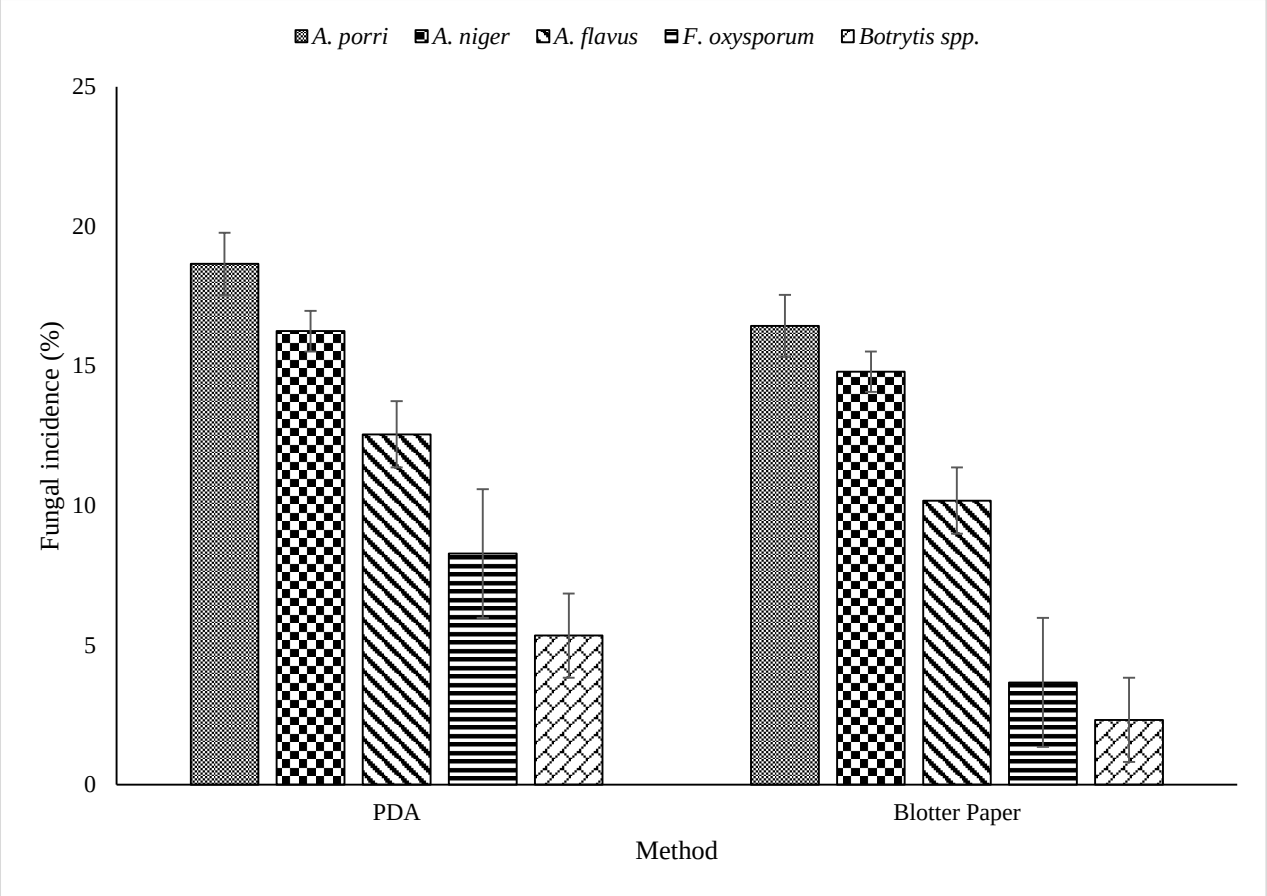

Fig.4. Graphical representation of mean values of for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Early Red.

During the evaluation of detection methods the fungus incidence was observed at higher level in PDA method (6.09%) as compared to Blotter paper method (4.55%) Table 4 shows that maximum fungal incidence was observed in case of PDA method as compared to blotter paper method. *Alternaria porri* showed maximum incidence while *Botrytis* spp. exhibited minimum incidence.

Table 4: Analysis of Variance for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Robina.

Source	DF	SS	MS	F	P
Fungus	4	386.671	96.6678	1377.88	0.0000
Method	1	17.772	17.7716	253.31	0.0000
Fungus × Method	4	5.469	1.3673	19.49	0.0000
Error	20	1.403	0.0702		
Total	29	411.315			

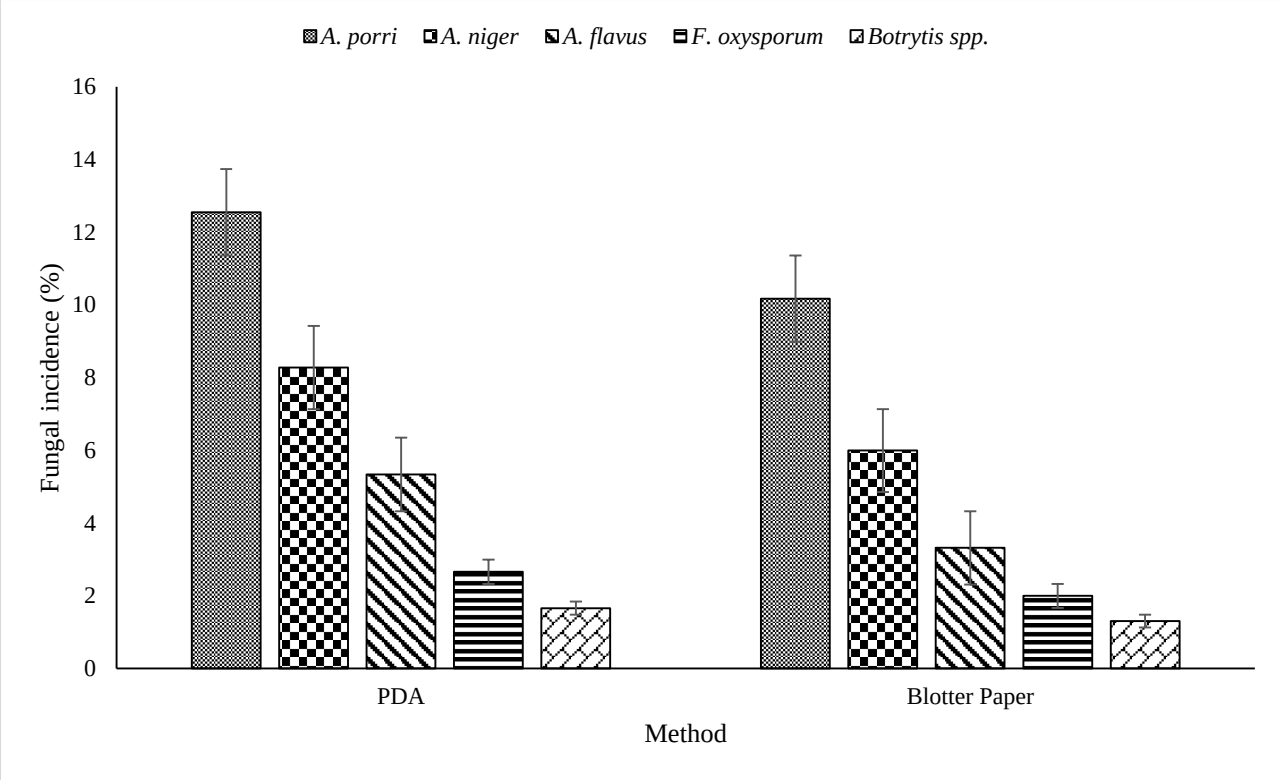

Fig.4. Graphical representation of mean values of for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Robina.

During evaluation of detection methods the fungus incidence was observed at higher level in PDA method (2.33%) as compared to Blotter paper method (1.45%) Table 5 shows that maximum fungal incidence was observed in case of PDA method as compared to blotter paper method. *Alternaria porri* showed maximum incidence while *Botrytis* spp. minimum incidence.

Table 5: Analysis of Variance for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Dark Red.

Source	DF	SS	MS	F	P
Fungus	4	42.4532	10.6133	14538.8	0.0000
Method	1	5.8344	5.8344	7992.37	0.0000
Fungus × Method	4	2.9845	0.7461	1022.08	0.0000
Error	20	0.0146	0.0007		
Total	29	51.2867			

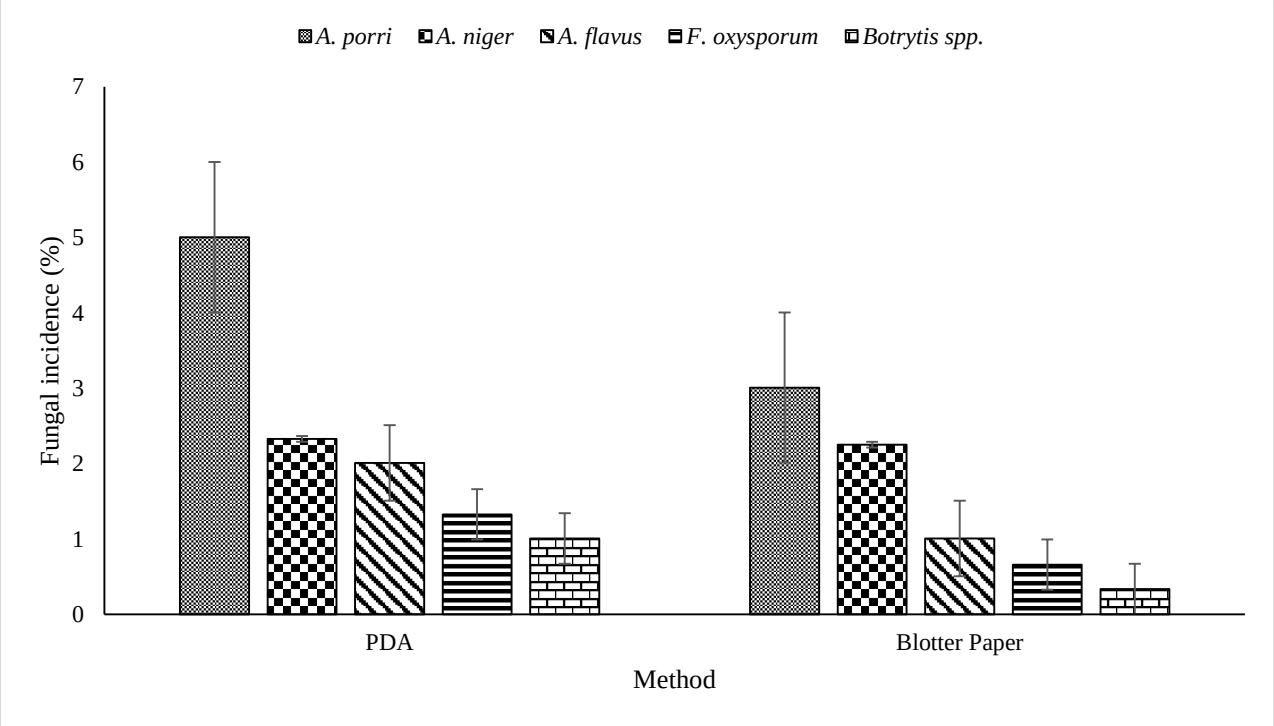

Fig. 5. Graphical representation of mean values of for detection of seed-borne fungi through blotter paper and agar plate method from onion variety Dark Red.

DISCUSSION

The dominant isolated fungi were *A. porri, A. niger, A. flavus, F. oxysporum, Botrytis spp.* from seed of different varieties of onions. These findings are in conformity with that of Shehu and Muhammad (2011) he also isolated above 5 fungi species from rotten bulbs of onion in addition to *A. fumigatus*. The findings of Aveling *et al.,* (1993) also in line with our results where they isolated the similar fungi from infected onion seeds in South Africa. The most encountered fungi was *A. niger* among the tested onion seeds. El-Nagerabi and Abdalla (2004) also reported that Aspergillus was commonly encountered genus from seeds of onion examined in Sudan. *A. niger* high dominance on seeds may attribute to its ability to use flowers vulnerability to pierce onion seeds (Sirois and Lorbeer, 1998).

In this study two methods were used Blotter Paper and PDA method, the fungus incidence was observed at higher level in PDA method (15.94%) as compared to Blotter paper method (13.47%). *Alternaria porri* showed maximum incidence while *Botrytis spp*. exhibited minimum incidence. The association of some seed-borne pathogens e.g. *A. niger*, *F. oxysporum* and *Botrytis sp*. are also known as the causal agents of post-harvest diseases of onion. These results are in line with the findings of Hayden *et al.,* (1994) and Sumner (1995) that few seed-borne pathogens may be carried up to storage. Hence, they contribute to onion post-harvest diseases. The role of seeds as one of the major source of disease development and fungal infections in the production of onion, cannot be over-emphasized.

Not all of the fungi isolated from the seeds of onion are pathogens in transfer of onion diseases. Seed-borne infections are only related if infected seeds transmit and germinate the pathogens to plant that

acts as primary source of disease in crops (Maude, 1980; Neergaard, 1979). However, few fungi are pathogens of known onion diseases. For example, *A. niger*, in extreme cases, inhibit root and shoot development because of pre-emergence damping-off (Gupta and Mehra, 1984).

CONCLUSION

Seed-borne infections are only related if infected seeds transmit and germinate the pathogens to plant that acts as primary source of disease in crops. Agar Plate and Blotter Paper methods are useful for the detection of various seed-borne fungi association in onion.

REFERENCES

1. Anonymous, 1996. Int. rules for seed testing. Seed Sci. Tech. 24:1-335.
2. Aveling, T.A.S., H.G. Snyman and S.P. Naude, 1993. Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. Plant Disease, 77:1009 – 1011.
3. Aveling, T.A.S., H.G. Snyman and S.P. Naude, 1993. Evaluation of seed treatments for reducing *Alternaria porri* and *Stemphylium vesicarium* on onion seed. Plant Disease, 77:1009 – 1011.
4. Barnet, H.L. and B.B. Hunter, 1972. Illustrated genera of imperfect fungi. American Phytopathological Society, St. Paul, Minnesota, 218 pp.
5. Booth, C. 1971. The genus Fusarium. Common Wealth Mycological Institute, Kew, Surry, England, 237pp.
6. Brewster, J.L., 2008. Onions and other Vegetable Alliums.2nd ed. CAB Int. North America.
7. Ellis, M. S. 1971. Dematiacious Hhypomycetes (C. M. I., Kew. Surrey, England), p. 608.
8. El-Nagerabi, S. A. F and A. H. M. Ahmed, 2001. The effect of black mould *Aspergillus niger*) on two Sudanese cultivars of onion. Tropical Sci., 41: 95 – 99.
9. El-Nagerabi, S.A.F and R.M.O. Abdalla 2004. Survey of seed borne fungi of Sudanese cultivars of onion, with new records. Phytoparasitica, 32:413-416.
10. El-Nagerabi, S.A.F and R.M.O. Abdalla 2004. Survey of seed borne fungi of Sudanese cultivars of onion, with new records. Phytoparasitica, 32:413-416.
11. FAO., 2011. Food and Agriculture Organization. (http://faostat.org/faostat/servlet). Accessed on 3rd January 2012.
12. Geremew, A., A. Teshome, T. Kasaye and C. Amenti, 2010. Effect of intrarow spacing on yield of three onion (*Allium cepa* l.) varieties at Adami Tulu agricultural research center (mid rift valley of Ethiopia). J. Hortic. For. 2:007-011, January, 2010.
13. Govindachari, T.R., G., Suresh, G., Gopalakrishnan, B. Banumathy, and S. Masilamani, 1998. Identification of antifungal compounds from the seed oil of Azadirachta Indica. Phytoparasitica. 26:109-116.
14. Gupta, R.P. and V. Mehra, 1984. Studies on the effect of fungal metabolites on viability and seedling vigour of onion seed. Indian J. of Plant Pathol., 2:100 – 102.
15. Hanelt, P. 1990. "Taxonomy, Evolution, and History." In Onions and Allied Crops, edited by Haim D. Rabinowitch and James L. Brewster, 1-26. Boca Raton, Fla.: CRC Press,
16. Hayden, N.J., R.B. Maude and F.J. Proctor, 1994. Studies on the biology of black mould (*Aspergillus niger*) on temperate and tropical onions 1. A comparison of sources of disease in temperate and tropical field crops. Plant Pathol., 43:562 -569.

17. Hayden, N.J., R.B. Maude and F.J. Proctor, 1994. Studies on the biology of black mould (*Aspergillus niger*) on temperate and tropical onions 1. A comparison of sources of disease in temperate and tropical field crops. Plant Pathol., 43:562 -569.
18. Howell, C.R. and R.D. Stipanovic, 1983. Gliovirin, a new antibiotic from Gliocladium virens, and its role in the biological control of *Pythium ultimum*. Canadian J. of Microbiol. 29:321-324.
19. Hussein, M., M. Hassan, A. Allam, K. Abo-Elyousr, 2007: Management of *Stemphylium blight* of onion by using biological agents and resistance inducers. – Egyptian J. of Phytopathol. 35:49–60.
20. Köycü, N.D. and N. Ozer, 1997. Determination of seed-borne fungi of onion and their transmission to onion seeds. Phytoparasitica, 25:25-31.
21. Lifshitz R., M.T. Windham and R. Baker, 1986. Mechanism of biological control of pre-emergence damping-off of pea by seed treatment with *Trichoderma spp*. Phytopathol, 76:720-725.
22. Lumsden, R.D., E., Garcia, J.A. Lewis and G.A. Frias, 1987. Suppression of damping-off caused by *Pythium spp.* in soil from the indigenous mexican chinampa agricultural system, Soil Biol. and Biochem. 19:501-508.
23. Mathur Kamlesh, R.B.S., Gurjar S.N. Shanna, and K. Sharma, 2007. Efficacy of fungicides, bioagents and plant extracts against pink root rot disease of onion induced by *Fusarium solani*. J. of Mycol. and Plant Pathol., 37(3).
24. Mathur, Kamlesh and S.N. Sharma, 2006. Evaluation of fungicides against *Alternaria porri* and *Stemphylium vesicarium* disease of onion in Rajasthan. J. of Mycol and Plant Pathol. 36:323-324.
25. Maude, R.B., 1980. Storage diseases of onions. In: H. D. Rabinowitch and J. L. Brewster (eds.), Onions and Allied Crops. CRC Press. Florida, USA. pp. 273 – 296.
26. Neergaard, P., 1979. Seed Pathology. Vol. 1. The Macmillan Press Ltd., London and Basingstoke.
27. Nene, Y.L. and B.N. Thapliyal, 1973. Fungicides in plant diseases control, 3rd edition, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, p.325.
28. Norman, J.C., 1992. Tropical vegetable crops. Arthur H. Stockwell Ltd. Ilfracombe, Great Britain.
29. Quenton, K., A.S. Theresa F.O. Walter P.R. Johon, V.D.W. Liana and S.S. Gardon, 2003. Mycoflora and fumonisin Mycotoxins Associated with cowpea (*Vigna unguiculata* L.) seeds. J. Agric. Food Chem. 51:2188-2192.
30. Raju, K. and M.K. Naik, 2007. Survey and Assessment for the Post Harvest Diseases of Onion in the North Eastern Karnataka, J. of Plant Pathol. 20:161-165.
31. Shehu, K. and S. Muhammad, 2011. Fungi associated with storage rots of onion bulbs in Sokoto. Nigeria. Int. J. of Mod. Biol., 1:1-3.
32. Sirois, K.L. and J.W. Lorbeer, 1998. Susceptibility of onion seed stalks and flowers to infection by *Aspergillus niger* Tiegh. In: Proceeding 7th biennial National Onion (and other Allium) Research Conference. California, December 10-12, pp. 353 – 360.
33. Sirois, K.L. and J.W. Lorbeer, 1998. Susceptibility of onion seed stalks and flowers to infection by *Aspergillus niger* Tiegh. In: Proceeding 7th biennial National Onion (and other Allium) Research Conference. California, December 10-12, pp. 353 – 360.
34. Steel, R.G.D., J H. Torrie and D.A. Dickey, 1997. Principles and procedures of statistics. A biometrical approach. 3rd Ed., McGraw Hill Co. New York.
35. Sumner, D.R., 1995. Diseases of bulbs caused by fungi – Black mold. In: H. F. Schwartz and S. K. Mohan (eds.).

Compendium of onion and garlic diseases. APS Press, St. Paul. Minnesota. pp. 26-27.
36. Sutton, B.C., 1980. The Coelomycetes. Common Wealth Mycological Institute, Kew, Surrey, England.
37. Tuite, J., 1969. Plant Pathological Methods, Fungi and Bacteria, Vol. I Burgess Publishing Co., Minneapolis, USA. p. 238.
38. Wainwright, M., 1979. Effect of fungicide on microbiology and biochemistry of soil-A review. Z. pfl. Eenahar. Bonahar. Bodenk, 140:587-603.
39. Wani, A.H. and T.U. Nisa, 2011. Management of black mold rot of onion. Mycopath. 9:43-49.
40. Weindling, R., 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathol. 24:1153-1179.
41. Wilhite, S.E., R.D. Lumsden and D.C. Straney, 1994. Mutational analysis of gliotoxin production by the biocontrol fungus Gliocladium virens in relation to suppression of Pythium damping-off. Phytopathology 84:816-821.