Faculty of Agriculture & Veterinary Medicine, Thamar University

Yemeni Journal of Agriculture & Veterinary Sciences

Available online at; WWW.YJAVS.COM

DOI: xxxx.xxxx

Isolation and identification of Fungi from the Animal Dung in Thamar-Yemen and Estimation of the Antagonistic Activity of *Papulospora* sp. against *Fusarium oxysporum*

M.A. Moghalles [1], S.M. Al-Bader [2]

1 College of Education - University of Thamar 2 College of Applied Science –University of Thamar,*Dhamar, Yemen Corresponding author e-mail; salahalbader@yahoo.com*

Article history:

Received

7 June 2014

Accepted

27 June 2014

ABSTRACT

This study was conducted in Thamar province – Yemen, and aimed to isolation and identification of fungi from the animal dung and testing the antagonism activity between the *Papulaspora* sp. isolated from the animal dung against *Fusarium oxysporum*. Twenty five dung samples of cows(12) and sheep(13) were collected from three different locations of Thamar province/ Yemen.By the moist chamber method, fifteen fungi were isolated (6 Hyphmycetes,5 Ascomycetes, 3 Zygomycetes,and 1Basidiomycetes); they include *Absidia corymbifera, Ascodesmis spherospora, Aspergillusflavus, A.niulans, Caetomium globusum, C.spirale, Coprinus* sp., *Kernia nitida, Mucor circinelloidis, Papulaspora* sp., *Phoma herbarum, Pithomyces* sp., *Rhizopus stolonifera, Sporormiella minima*..The species richness of samples was different according to the samples location, as well as to the type of samples. *Papulaspora* sp. was selected as antagonist against *Fusarium oxysporum* ,the results revealed that the growth in the medium of the potato dextrose agar showed a high effect of Papulaspora isolate on the radial growth of pathogenic isolate of *F. oxysporum* .At the same time, *Papulaspora* sp did not affect on neither the seeds germination% nor on seedlings growth of tomato. It is worthy to mention that the recent study was regarded as a new investigation about antagonism efficacy of Papulaspora.

Keywords: Coprophilous fungi, Yemen ,Antagonism, Papulaspora, Fusarium,

INTRODUCTION

Dung of herbivores is rich substrate and suitable for fungal growth, and the group of which inhabit t animal excreta known as coprophilous fungi .These fungi have an important role in the decomposition of herbivorous dung (Bell,2005). Lodha (1974) mentioned that the true coprophilous fungi should be passed through animal guts to develop their reproductive structures. Some of them do not need such rout and known as (facultative coprophilous). Coprophilous fungi were regarded as important biological control agents, and a source of enzymes and antibiotics.(Bills et al, 2013; Al-deen et al 1990; Magnelli et al,1996 ;Lynd et al 2002). Their diversity were investigated according to temporal and spatial changes(Abdullah,1982; Richardson ,2001-a; Kruys and Ericson,2008;Thoke et al,2007).

A little is known about this group of fungi in Yemen ,and the current study was carried out to record the coprophilous fungi on cows and sheep dung in Thamar province, and to test the antagonistic activity of *Papulospora* sp. isolate against *Fusarium oxysporum*

MATERIALS AND METHODS

Twenty five dried dung samples of sheep(12) and cows(13) were collected from three different locations in Thamar province during the period 5/3 to 25/3/2013.These locations were I-Rasabah (a breeding station of cows in Thamar), II- Al-Rabo'a market (a central market for domestic animals in Thamar),and III-Thamar Al-garen (a rural site for natural grazing).

The moist chamber culture method was used to induce fungal growth at room temperature, and natural light (Bell,2005). The plates were cheked continuously to moist them by distilled water , and to observe the fungal growth. All the developing fungi were identified either directly from the tested samples, or after preparing a pure cultures .Identification of fungal isolates was done after (Domsch et al,1979 ; Watanabae , 2002). The pathogenic fungus (*Fusarium oxysporum*) was obtained from an infected plant of tomato (*Lycopersicon esculantum* L.).It was isolated on PDA and was identified according to(Watanabae , 2002). Dual culture test was followed to evaluate the antagonistic activity between the antagonists(*Papulaspora* sp.) and *F.oxysporum* (Adebola and Amid,2010). The Percentage of inhibition was calculated using the following formula :

Inhibition% = C- C/T X 100

(**C**= radial growth of pathogen in control) (**T**= radial growth of pathogen in treatment) (Sengh et al 2002)

Effect of *Papulaspora* sp. on germination % of tomato seeds and on seedlings growth was tested in (1kg) pots. Two pots were used, the first contains sterile soil contaminated by *Papulaspora* sp. ,while in the second pot, a sterile soil only was used as control. Twenty seeds were planted in each pot and equal amounts of sterile water were added to the pots during the experiment period.

RESULTS AND DISCUSSION

Fifteen fungal isolates were recorded from dung samples from three locations in Thamar province (Rasabah, Al-Rabo'o market, and Thamar al-garen) (Table-1). The isolated fungi include Ascomycetes(5) ,Hyphomycetes(6) , Zygomycetes(3) , and Basidiomycetes(1).

Table 1. Coprophilous fungi and their locations, dung types, species diversity Location and taxonomic groups. (R=Rasabah) (A=Alrbo,a market) (T=Thamar Al-garen)

Fungi	Location	Dung sample	Taxonomic group	species diversity /Location
Aspergillus flavus	R	Cow &sheep	Hyphomycetes	
A.fumigatus	R	Cow	Hyphomycetes	
Ascodesmis spherospora	R	Cow	Ascomycetes	**0.22**
Chaetomium globosum	R	Cow	Ascomycetes	
C. spirale	A&T	Cow& Sheep	Ascomycetes	**0.11**
Coprinus sp.	A	Cow	Basidiomycetes	
Kernia nitida	A	Sheep	Ascomycetes	
A.nidulans	A	Sheep	Hyphomycetes	
Phoma herbarum	A	Cow	Coelomycetes	**0.34**
Pithomyces chartarum	A	Cow	Hyphomycetes	
Sporormiella minima	A	Cow	Ascomycetes	
Mucor circinloides	T	Cow	Zygomycetes	
Rhizopus stolonifera	T	Sheep	Zygomycetes	**0.11**
Papulaspora sp.	R&A	Sheep	Hyphomycetes	
Absidia corymbifera	R&A	Sheep	Zygomycetes	**0.22**

Nine fungi were represented from the samples of (Al-Rabo'a) ,six fungi from Rasabah, and only three isolates from (Thamar al-garen) .The type of feeding may affect on the species diversity , this is in conformity with previous studies (Ebershon and Eicker,1992; Carreta et al 1994) . In Al-Rabo,a market, the animals were brought weekly from countryside around the city of Thamar , and this may arise probability of species diversity to (0,34).On the other hand the low species diversity in Thamar Al-garen (0.11) may due to the type of natural feeding available during the sampling period (dry &cold).Previous studies have also shown that the type of feed that the animals ingest affects on the mycoflora of herbivorous dung (Abdullah

,1982 ;Thoke et al,2007). The environmental factors as well as the low number of collected samples, may also caused the decreasing in a total species which were recorded in the present study, Richardson (2001-b) explained that a high number of fungi were recorded in the coincidence of increasing in the number of samples.

Twelve species were recorded from cows samples versus six species from sheep's dung (table-1). Richadson (2001) explained the highly significant differences among mycobiota of different dung types.

Absidia, Aspergilli, Mucor, Papulospora, Pithomyces, Phoma and, Rhizopus which developed on the dung samples were considered as opportunist and not a restricted coprophilous fungi and they arrived on the dung after its voidance. These genera were previously isolated from dung in several studies (Abdullah,1982; Elshafie , 2005;de Azevedo, 2011; Yadav, 2011),and they probably contaminated dung from the air and soil.

In this study, the hyphomycetes (*Papulaspora* sp.) was selected as antagonistic agent among the other isolates because of its single development on sheep pellets . Moreover it's easily purification, and grew too rapidly on PDA medium. The antagonistic test between *Papulaspora*sp.and *Fusarium oxysporum* showed a reduction in mycelia growth of the pathogen(75% - a mean of three replicates) (Figur.1).The efficacy of Paulaspora which was recorded here exceeded that was previously mentioned of *Trichoderma* spp. (Mansoor et al 2012 ; Kahkashan and . Bokhari 2012).

Examining the damage that may be caused by Papulasporasp showed that neither seed germination% was affected nor seedlings growth until two weeks after emergence . Several articles were mentioned the antagonistic activity of saprophytic fungi and parasitic fungi (Suarez-Esterella et al 2007; Hydri and Pessaraki 2010) , but the information about Papulaspora in this field was very rare, and the isolate that was recorded here needs farther studies to be applicable.

Figure.1: Five days old of: left-*F.oxysporum* the pathogen , right-antagonism between *F.oxysporum* and *Papulaspora* sp.

References

Abdullah, S. K. 1982 .Coprophilous mycoflora of different dung types in southern desert of Iraq.Sydowia vol. 35.

Al-deen, S.J.,E.K Al-Habeeb, and S.K Abdullah ,. 1990 .Cellulytic activity of coprophilous fungi. Cryptogamic Botany vol.2,no.1: pp25-29

Bell, A. 2005. An illustrated guid to the coprophilous Ascomycetes of Australia.CBS Biodiversity series 3.

Bills, G F. , J.B. Gloer, and An Z. 2013. Coprophilous fungi: Antibiotic and function in an underexplored arena of microbial defensive mutualism.Curr.Opin.Microbiol. 16:549-565.

Caretta, G. and E. Piontelli .1996. Coprophilous fungi from confined deers in Pavia (Lombardia, Italy) Bol. Micol. ,11:41–50.

Domsch, K. H, W. Gams, and T. Enderson. 1980. Compenedium of soil fungi.vol. 1,pp859.Academic press.London.

Dix, N. J and J. Webster. 1995. Fungal Ecology. Chapman & Hall; London. P: 549.

Ebersohn, C. and A. Eicker. 1997. Determination of the coprophilous fungal fruit body successional phases and the delimitation of species association classes on dung substrates of African game animals. *Bot. Bull. Acad. Sin.* 38:183-190.

Elshafie, A E. 2005. Coprophilous mycobiota of Oman. Mycotaxon 93:355-357.

Hydari, A. and M. Pessaraki .2010. A review on biological control of fugal plant pathogens using microbial antagonists .J. of Biological Sci.vol.10,4:273-290.

Kahkashan, P. and N. A. Bokhari .2012. Antagonistic activity of *Trichoderma harzianu*m and Trichoderma viride isolated from soil of date palm field against *Fusarium oxysporum* .African J. of Micro. Res.Vol. 6(13), pp: 3348-3353.

Kruys, A. and L. Ericson. 2008. Species richness of coprophilous ascomycetes in relation to variable food intake by herbivorous. Fungal divers 30:73-81.

Lodha , Eds., C. H. Dickinson, and G. H. P ugh . 1974. Decomposition of digested litter In Biology of plant litter decomposition. Academic Press, New York. pp: 213 – 241.

Lynd L. R, J. W. Paul , and W. H van Zyl .2002. Microbial cellulose utilization .Fundamental and biotechnology.

Microbiology and molecular biology review 66,pp506-577.

Magnelli, P.A., M. Ramos, and F. Forchiassin . 1996 . Factors influencing cellulose production by *Saccobolus saccoboloides*,Mycologia, Vol. 88, No. 2, pp:249-255.

Mansoor, A. L ,R. W. Mohd , A. S Subzar, S. I Sanjay , and M. Suliman Dar .2012. Antagonistic Potentiality of *Trichoderma harzianum* Against *Cladosporium spherospermum*, *Aspergillus niger* and *Fusarium oxysporum*. Journal of Biology, Agriculture and Healthcare Vol 2, No.8: 72-76.

Richardson, M. J.2001-a. Diversity and occurrence of coprophilous fungi. Mycological Res.vol 105,issu 4,pp: 387-402.

Richardson, M.J. 2001-b. Coprophilous Fungi from Brazil. Braz. Arch. Biol.Technol 44(3) pp: 283–28.

Sengh, R.S., B.K. Upadhyay, B. Rai, and Y.S Lee .2002. Biological control of *Fusarium* wilt disease of pigeonpea. Plant Pathol. J., 18: 279-283.

Suarez-Esterrella, F. , C. Varagas-Garica , M. J Lopez, C. Capel,and J. Moreno .2007. Antagonism bacteria and fungi from horticultural compost against *F.oxysporum* f. sp. *melonis.*Cropprotection.vol 26,1, pp :46-53.

Thoke, R. B, L .N Nair, and B.A Kore . 2007. Studies of coprophilous fungi. Bionfolet 4(3).pp: 256-258.

Watanabae, T.2002.Pictorial atlas of soil and seed fungi. Morphologies of cultured fungi and key to species.CRC press, pp:479.

Yavad, S. K. 2011. Studies on diversity, ecology, and activity of coprophilous fungi from Goa and neighboring regions of Mahrashatra and Karnataka-India. PhD thesis. Goa university, India .pp: 194.