New way to increase the efficiency of *Aspergillus niger* and *Penicillium chrysogenum* as biofertilizers on wheat by using extraction of okra bark and Turmeric .

Dewan , M.M. ; Alwan , K.F. and Alsahaf , F.H.

Faculty of Agriculture / University of Kufa - IRAQ

Abstract

This study was conducted to increase the spores adhesion of biofertilizers (*Aspergillus niger* and *Penicillium chrysogenum*) on wheat grains by using okra bark (*Abelmoschus esculentus*) and Turmeric (*Curcuma zedoaria*) extractions as warning color and their effect on growth of pathogenic fungi (*Pythium aphanidermatum* and *Rhizoctonia solani*) , biofertilizer fungi and wheat growth .

The extraction of okra bark reduced the radial growth of *R.solani* and *P.aphanidermatum* to 7.4 , 6.1 cm . compared with 8.17 , 7.5 cm . on potato extraction respectively . it was found the okra bark extraction increased the sporulation of *A.niger* and *P.chrysogenum* to 7.0 , 8.33(10^5) compared with 5.76 , 6.86(10^5) on potato extraction respectively too .

The okra bark extraction promoted the lengths of shoot and root of germinated grains of wheat to 5.4 and 2.4 cm and nomber of rootlets 5.3 respectively compared with 2.0 , 0.8 cm and 3.0 in potato extract .

Also , the mixture of okra bark and Turmeric extraction inhibited the radial growth of *P.aphanidermatum* and *R.solani* while increased the radial growth and sporulation of biofertilizers fungi (*A.niger* and *P.chrysogenum*) .

The spore suspension in okra bark and Turmeric mixture increased the carried spores on wheat grains for *A.niger* and *P.chrysogenum* to 169.23 and 217.65 % respectively . while the percentages of adhesive spores on dry grains were 136.76 and 115.79 % respectively too.

Key words : *A.niger* , *P.chrysogenum* , Okra , Turmeric , extraction , biofertilizers , wheat grains

Introduction

Biofertilizer are substance that contain living micro-organisms , and they colonize the rhizosphere of plant and promote its growth by increasing the a vailability of nutrients and the secondary metabolites of biofertilizers agents

contain on indol acetic acid (IAA) (Dewan , *et al* 1994) or have ability to producing plant hormones which are important in control root pathogens by stimulating systemic resistance (Xiao , *et al* ; 2008). In general biofertilizer agent including living cells and their exudates in soil which have ability to make plant nutrients from unusable to usable form through some biological process . (Ismail , *et al* , 2014) . *Aspergillus niger* , *A.fumigatus* and *Trichoderma hamatum* promoted the growth and yield of wheat (Al-Taie , *et al* 2016).

Aspergillus niger and *Penicillium notatum* promoted the growth and yield of ground nut when the soil amended with them . *P. notatum* incressed dry weight , yield , protien and oil of ground nut . (Malviya , *et al* , 2011).

The biofertilizers agent acte as biocontrol agent at the same time such as *Trichoderma harzianum* (Gisalberti *, et al* , 1990) , and sterile red fungus (Dewan and sivasithamparam , 1990). The sterile red fungus promoted the growth of wheat plants and increased grain yield when added in high inoculum to soil , in the same time the fungus reduced the severity of take – all disease on wheat (Dewan and sivasithamparam , 1989).

The advantages of biofertilizers are make microbial and chemical balance , health crop products and eco-friendly . Biofertilizers can increase the crop yield 20-30% , in addition to biofertilizers low cost and renewable compared with synthetic fertilizers (Pal , *et al* , 2015).

Materials and Methods

1-Effect of some plant extraction in growth and sporulation of fertilizers and pathogenic fungi

The study was conducted to choice adhesive extraction from some plants and the Turmeric used to coloring the wheat grains during the dressing them by spores of biofertilizers fungi.

Slices of potato tuber , *Aloe vera* leaf , okra bark , sow thistle , Turmeric , were dried in oven at 60 °c for 48h , after that ground individually . 50 , 23 , 20 , 17 , and , 32 g of each plant powder respectively were added to 1L distilled water , then were boiled to 15 min . and filterated by five layers of smoth mousseline cloth . 15 g sucrose /L added to the filteration . The filterated liquid divided to two parts , the first , dissolved in it 17 g /1L agar to use it as soild medium while the

second with out agar (Broth medium) . The soild and broth media autoclaved at 120 °c , 15 psi for 20 min . 20 ml of sterilized agar medium was poured in each plate to test the growth and sporulation of

biofertilizers and pathogenic fungi , while the broth medium used to germinate wheat grains and spore suspension .

2-The radial growth and sporulation of biofertilizers and pathogenic fungi on agar medium amended with plant extractions .

The biofertilizers fungi : *Aspergillus niger* , *Penicillium chrysogenum* and pathogenic fungi : *Pythium aphanidermatum* , *Rhizoctonia solani* were cultured in plate center by 0.5 dim.cm plug on agar medium which prepared in (1) . The inoculated plates were incubated at 25 °c ± 2 , the redial growth of *p.aphanidermatum* and *R.solani* calculated after 2d. , while the biofertilizers fungi *A.niger* and *P.chrysogenum* after 5d.

The spore formation of biofertilizers fungi were measured by taking one plug (1cm.dim.) from each colony at 5d . old which growing on plant extraction media in 9 ml sterilized distilled water , and serial dilutions were done to 10^{-6} . One ml of this concentration put in plates and 15ml of P.S.A. pured on them , Three

replicates were done to each treatment . The plates incubated at 25 °c ± 2 for 24 h . The spores density were calculated by the formula : No.of colonies × 10^6 (clark , 1965).

3- Effect of plant extraction on germination and seedling growth of wheat grains .

The plant extractions (broth medium) as in (1) were used , 5 ml of each plant extraction individualy put in plate on sterilized filter paper . 10 sterilized grains were cultured in each plate . The plates placed in germinater on 25 °c ± 2 , 12 h . light : 12 h . dark . The nomber of germinated grains , nomber . of root lets , length of shoot and root were taken after 5 d.

4- The Inoculation apparatus

The inoculation apparatus was designed and manufactured by the authors of this research. The purpose of this manufacturing to dressing the wheat grains by spores , extraction and exudates of biofertilizers fungi in closed system to prevent the biopolution .

The parts of apparatus are :

A- Graduated plastic Tube (G.P.T.).

The dimensions of G.P.T. are 20 cm. length 3 cm. diam. with upper and bottom holes.

B- Narrow plastic Tube (N.P.T.).

N.P.T. is 36 cm. length , 0.5 cm. diam. Fixed on the bottom hole in G.P.T. and longitudinal extends (23 cm) on upper surface of grains container , with small nozels opened inside the grains container.

C- Grains container (G.C.).

The G.C. is barrel shape with two holes . One on the upper surface to enter the grains while the second hole on the bottom surface of G.C. to exit the dressed grains .

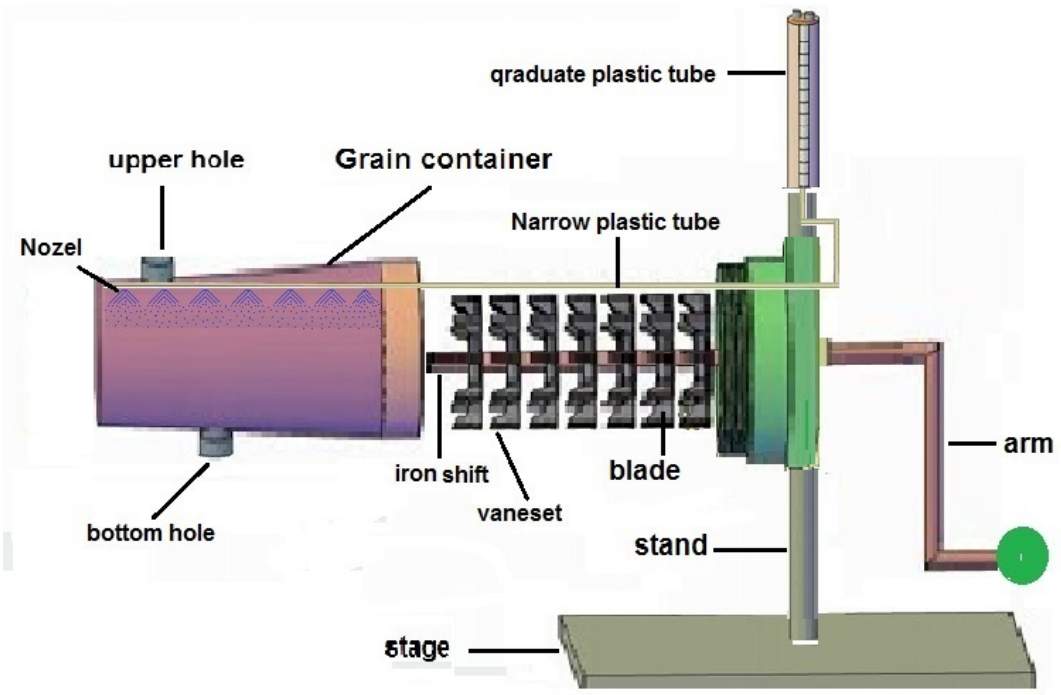

Fig.I Inoculation apparatus

D- Iron shift (I.S.)

.length , 1 cm. diam. and The iron shift elongates in inside the G.C. 32 cm connect with arm in outside the G.C.

E- Vane set (V.S.)

Seven vanesets (7 cm. diam. 7 cm. width.) were fixed on the iron shift .The distance between the V.S. and another one is 2.2 cm.

F- Iron Stand (44 cm. length and 0.75 cm. diam.) and stage (36 cm. length , 28 cm. width and 2.0cm. thickness)

5- Determination of adhesive spores of biofertilizers on wheat grains by using okra bark and Turmeric extractions .

10 plugs (1cm.dim.) from colony at (5 d. old) of *A. niger* and *P.chrysogenum* were added to 90 ml of okra bark and Turmeric (for warning color) extraction in beaker to each fungus . The beakers shaked to 5 min . The plugs removed by filteration through five layers of sterilized mousseline cloth .

250 gm . of wheat grains were put in the grain container of inoculation apparatus . 50 ml of spore suspension in mixture of okra and Turmeric extraction or potato extraction (control) put individualy to each biofertilizers in graduated plastic tube .

The mixture of spore suspension and Turmeric extraction pushed throw the narrow plastic tube to nozels . The wheat grains treated by spores and warning color (Yellow) by the movement of the shift arm until all the grains were saturated by the spore suspension . The grains pull out and put on sterilized filter paper to remove the free water , after that dried and stored . To determine the carrying spores on wettish grains , 1gm of treated grains was added to 9 ml sterilized water . The spore density culculated by dilution method .

The adhesive power of spores on the treated wheat grains measured by tacking 5 gm of dry grains and put in disposible syring (size 60 ml) .

The piston of syring pushed strongly 5 times on P.S.A. medium in plates . The formed colonies from dry treated grains by okra or potato extraction were culculated .

Results and Discussions

1- The result showed (Table 1) that the extraction of okra bark reduced the radial growth of pathogenic fungi (*R.solani* and *P.aphanidermatum*) to 7.4 , 6.1 cm. compared with 8.17 , 7.5 cm. on potato extraction respectively . while no negative affect on the radial growth of biofertilizers agents (*A.niger* and *P.chrysogenum*) . In the same time the the okra extraction increased the sporulation of *A.niger* and *P.chrysogenum* to 7.0 , 8.33 (10^5) compared with 5.76 , 6.86 (10^5) on potato extraction. The increasing and reducing the fungal growth or sporulation depend on the released compounds from medium substance by biodegradation . it was found that *A.niger* has high cellulase activity while *Penicillium* sp . has high ability in lignin degradation (Subowo , 2009). Reduction growth of pathogenic fungi and increasing growth and sporulation of biofertilizers agents by okra bark extraction encourage to use it as adhesive substance for biofertilizer spores on wheat grains.

Table 1: Effect of powder extraction of potato , okra bark , *Aloe vera* and Sow thistle on radial growth of *R.solani* and *P.aphanidermatum* and growth and sporulation of *A.niger* and *P.chrysogenum*.

Culture	Fungal radial growth (f.g.) / cm	Fungal sporulation (10^5)

media(c.m.)	*P.aphani.*	*R.solani*	*A.niger*	*P.chryso.*	Mean	*A.niger*	*P.chryso.*	Mean
Potato	8.17	7.5	1.86	1.44	4.70	5.76	6.86	4.70
Okra	7.4	6.1	1.88	1.50	4.20	7.00	8.33	4.20
Aloe vera	7.5	5.6	1.74	0.82	0.09	3.70	5.23	3.90
Sow thistle	8.93	7.13	2.16	1.04	4.80	2.20	5.83	4.80
Mean	8.02	6.58	1.91	1.20		4.67	6.56	
L.S.D. $_{0.05}$	**c.m. = 0.151 ; f.g. = 0.151 ; Int. = 0.302**				**c.m. =0.708 ; f.g. = 0.501; Int. = 1.002**			

2- It was found (Table 2) the extractions of okra bark , *Aloe vera* , and sow thistle increased the shoot , root lengths and nomber of rootlets of wheat , but the okra bark extraction was more efficiency in growth parameters , whereas reached to 5.4 , 2.4 cm. and 5.3 respectively compared with 2.0 , 0.8 cm. and 3.0 in potato extraction respectively too . This result added other benefit to the biofertilizers from promoting the growth of wheat seedling by okra bark extraction .

Table 2 : effect of powder extraction of potato , okra bark , *Aloe vera* and sow thistle on seedling growth of wheat for 5 d. after planting

Extraction	Length (cm)		No.of rootlets (nr)
	Shoot(s)	Root(r)	
Potato	2.0	0.8	3.0
Okra	5.4	2.4	5.3
Aloe vera	3.4	1.9	3.6
Sow thistle	2.3	1.2	3.6
L.S.D. $_{0.05}$	**s = 0.932 ; r = 0.403 ; nr = 0.941**		

3- The results (Table:3) indicated that the mixture of okra bark and Turmeric inhibited the radial growth *R.solani* and *P.aphanidermatum* to 2.2 , 7.7 cm. compa in potato extraction respectively .

Fig. 2 wheat grains treated with extraction :

A- Potato B- okra bark & Turmeric

Fig. 3 *R.solani* growing on

A- Potato B- okra bark & Turmeric

while the growth and sporulation of *A.niger* and *P.chrysogenum* increased to 1.65 cm , 8.7 (10^5) and 1.09 cm , 14 (10^5) in mixture of okra bark and Turmeric compared with 1.4 cm , 5.47 (10^5) and 0.84 cm , 11.33 (10^5) in potato extraction respectively . The reduction of mycelial growth of *R. solani* and *P.aphanidermatum* may be retain to relase some specific compounds from extractions of okra bark and Turmeric affected on growth of these fungi . Turmeric has been found effective for controlling mycelia growth of *Fusarium oxysporum* (Singh and Maurya , 2002) . while the increasing the growth and sporulation of *A.niger* and *P.chrysogenum* on mixture of okra bark and Turmeric extraction due to dissolve inorganic phosphorus and production organic acids (Akintokun *et al* , 2007) .

The inoculation apparatus proved a high efficiency in dressing the wheat grains by biofertilizers spores in closed system to reduce the biopolution . This apparatus also protects the agricultural workers from the harmful fungal spores which reach for them during the respiration system .

Table:3 Effect of mixture of okra and Turmeric extraction in radial growth (cm) of pathogenic fungi (*P.aphanidermatum* and *R.solani*) and radial growth and sporulation (10^5) of fertilizers fungi (*A.niger* and *P.chrysogenum*)

Culture media (c.m.)	Fungal radial growth (f.g.) / cm					Fungal sporulation (f.s.)		
	P.aphani.	*R.solani*	*A.niger*	*P.chryso.*	Mean	*A.niger*	*P.chryso.*	Mean
Potato extraction	8.43	7.66	1.40	0.84	4.58	5.47	8.70	7.08
Mix.okra &Turmerc	7.70	2.20	1.65	1.09	3.16	11.33	14.00	12.67
Mean	8.06	4.93	1.52	0.96		8.40	11.35	
L.S.D. $_{0.05}$	c.m. = 0.23 ; f.g. = 0.33 ; Int. = 0.47					c.m. = 1.46 ; f.s. = 1.46 ; Int. =		

		2.06

4- The results appeared (table :4) the mixture of okra bark extraction increased the carried spores of *A.niger* and *P.chrysogenum* after grain dressing by spore suspension in okra bark and Turmeric mixture . The population density of spores were 3.5 and 5.4 (10^5) /gm grains in okra bark and Turmeric suspension for *A.niger* and *P.chrysogenum* respectively compared with 1.3 and 1.7 (10^5) in potato suspension respectively too , with increasing percentages 169.23 and 217.65% for *A.niger* and *P.chrysogenum* respectively . This result also is very important to use okra bark extraction as adhesive substance and Turmeric as warning color for biofertilizers *A.niger* and *P.chrysogenum* .

Table :4 Effect of extraction of okra and Turmeric mixture on the carried and adhesive spores on wettish wheat grains and dry grain.

Extraction (E)	**Fungi**				
	A.niger		***P.chryso.***		***Mean***
	spore density (10^5)	**% increasing**	**spore density (10^5)**	**% increasing**	
Potato extraction	1.30	-	1.70	-	1.50

Mix. okra &Turmeric	3.50	169.23	5.40	217.65	4.48
Mean	2.40		3.50		
L.S.D. $_{0.05}$	**E = 0.34 ; fungi = 0.34 ; Int. = 0.48**				

5- Also , It was found (table:5) the adhesive spores on dry wheat grain increased to 136.76 and 115.79 % for *A.niger* and *P.chrysogenum* respectively . The increasing of adhesive spores on the wheat grains due to the okra bark contains on the glue compound . This result is very important because the okra bark extraction increase the spore density on the wheat grains and reduce the bio polution by fungal spores acording to the adhesive substance . The okra bark extraction make the biofertilizer more eco-friendly .

Table: 5 Number . of released spores of *A.niger* and *P. chrysogenum* from dry dressed grains in suspension spores in okra bark and Turmeric extractions

Extraction (E)	**Fungi**				
	A.niger		***P.chryso.***		**Mean**
	released spores	**% adhesive increasing**	**released spores**	**% adhesive increasing**	

Potato extraction	858.67	-	1093.33	-	976.00
Mix. okra &Turmeric	362.67	136.76	506.67	115.79	435.00
Mean	611.00		800.00		
L.S.D. $_{0.05}$	**E = 82.70 ; fungi = 82.70 ; Int. = 117.00**				

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