Antifungal Activity of Some Plant Extracts Against *Rhizoctonia solani*, the Causal Agent of Damping-off Cotton Disease

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ABSTRACT: Cotton (*Gossypium barbadense* L.) is one of the strategic farm crops which is widely cultivated and traded across the world. *Rhizoctonia solani* Kuhn is the most important fungal disease that caused pre- and post-emergence damping-off, sore shin and root rot of cotton seedlings. The present study was carried out at Faculty of Agriculture, Saba Basha, Alexandria University, Egypt during the period of 2016 to 2018. Fifteen isolates were collacted from Alexandria, Gharbia, Beni Suef (Sids-Giza 95), El-Beheria, Dakahlia, Kafr El Sheikh (Qelen) and Monufia (Beer alsaba) governorates. Data showed that the isolate from Kafer El-Shikh was the highest percentage (98%) forward by Monufya (94%) then Damanhor by 82%. While Giza 92 showed lowest values recorded to by 16.66%, Giza 45 and 94 by average was almost 17%. Data indicated that Giza 80 was more tolerant (23.125%) comparing with the susceptibly one Giza 93 (60.648%) and both Giza 87 and 92 showed moderate tolerant to fungal. Turmeric as extract plant showed the lowest fungal growth (3 cm) in Kafer El-shikh and (2 cm) in Giza 45 isolates. That mean this save natural material could be useful to control the *R. solani*.

Key words: Cotton seedlings*, R. solani,* plant extracts, antifungal activity

INTRODUCTION

Cotton (*G. barbadense* L.) is one of the strategic farm crops which is widely cultivated and traded across the world. Cotton seedling diseases are a worldwide problem which caused by a complex of soil-borne organisms. These organisms are found in all cotton producing areas in Egypt and include *R. solani* and *Fusarium* spp. *R. solani* Kuhn. The anamorph of *Thanatephorus cucumeris* (Frank.) Donk, causes seedling blight, pre- or post-emergence damping-off, sore shin and root rot of cotton seedlings (Asran *et al*., 2005). *R. solani* colonizes soft tissues and forms infection cautions. From these cautions, the fungus penetrates the epidermis and destroys plant cells (Watkins, 1981). (Disfani and Zangi., 2006), damping-off is one of the most important diseases in Egypt where cotton is grown. This disease caused seedling damping-off and decreasing the cotton production. Without fungicides treatment diseases was epidemic in cold and moisture soil. There are several soils borne pathogens in cotton seedlings such as *R. solani* AG-4 that was the most frequently isolated causal pathogen of the disease. Some isolates of *R. solani* reduced emergence and caused root discoloration on cotton seedlings during pathogenicity studies (Moustafa *et al*., 1995). The widespread soil borne pathogen *R. solani* is responsible for serious damage to many economically important agricultural and horticultural crops as well as trees worldwide (Grosch *et al*., 2006). Maurice *et al.* (2010) obtained twenty-eight isolates of *R. solani* from cotton seedlings and twenty-three isolates from other hosts; eight from peanut, five from chickpea, two from each of flax, tomato and watermelon and one from each of potato, cantaloupe, pepper and lupine. Manju rani *et al*. (2013) reported that *R. solani*

anastomosis groups (AGs) 7 and 4 are proven to be the most common pathogenic fungal strains on cotton crop.

Chemical fungicides are commonly used successfully for control of *R. solani* (Khan *et al*., 1998). The persistent, injudicious use of chemicals was discouraged owing to their toxic effects on non-target organisms, the undesirable changes they inflict upon the environment (Arcury and Quandt 2003) and due to the development of resistant strains of pathogens against various chemical fungicides (Deising *et al*., 2008). Keeping in view the drawback of chemical control of plant diseases, the use of plant extracts in the control of plant diseases is gaining importance. Various plant products like plant extracts, essential oils, gum, resins… etc. were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Pawar and Thaker 2006; El-Mougy and Alhabeb 2009; Fawzi *et al*., 2009). The main reasons for using plant extracts as antifungal agents is their natural origin and low chance of pathogens developing resistance. They may have a minimum adverse effect on physiological processes of plants and less environmental hazards compared to their synthetic alternatives, being plant products are easily convertible into a common organic material (eco-friend) (Gnanamanickam 2002).

The main objectives of the present research are to isolate *R. solani* from infected cotton seedling from different areas in Egypt and to study the effect of different plant extracts on growth of *R. solani* under *in vitro* conditions.

MATERIALS AND METHODS

The present experiments were carried out at the both Plant Protection and Agricultural Botany Departments, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt. These studies were conducted during 2016 - 2018.

Isolation of the pathogen

R. solani was isolated from naturally diseased cotton plants with typical symptoms of root-rot and damping-off. Plant samples were collected from different Egyptian governorates (Alexandria, Monufia, Kafr El Sheikh, Gharbia, Beni Suef, Dakahlia and El-Beheria). Purification of the isolated fungi was done using the hyphal tip technique. Fifteen isolates were identified as *R. solani* based on the cultural properties, morphological and microscopical characteristics as described by Sneh *et al*. (1991).

Pathogenicity test of *R. solani*

It was carried out to determine the pathogenic potential of different isolates of *R. solani*, and the most aggressive isolate. Sterilized pots (15 cm diameter) were filled with disinfested soil. Inocula of *R. solani* isolates were prepared by growing each isolate in 250 ml conical flask. Each flask was filled with 100 g of barley grains, 20 ml of tap water and 5 g clean sand, then autoclaved for 45 mins. Flasks containing sterilized grain medium were

inoculated with the isolates and incubated at 25±2°C for 10 days (Whitehead, 1957). Soil infestation was done by mixing the inoculum of each isolate with the upper layer of the soil at the rate of (5 g /pot) potential inoculum. The disease incidence (pre- and post-emergence damping-off) was observed by recording the number of unmerged seeds 15 days after sowing as well as number of dead seedlings 45 days after sowing (Bheemaraya, 2014).

Preparation of plant extracts:

Six different plants namely pomegranate, wheat bran, banana, liquorice, cinnamon and turmeric were used in this study. Plant extracts were obtained according to the method described by Bernhoft (2010) with some modification.

The plant materials were sterilized by ethyl alcohol 100% and dried in oven at 30°C for 24 hours. They were then finely grinded to powder. 6 grams of each plant material in powder form. The mixture with ethyl alcohol and distilled water (20: 80, 50: 50 v/v) and incubated in water bath at 35°C for 30 mins, then repeated for 3 days to complete extraction. The blank was taken by using distilled water instead of ethyl alcohol and following the same procedure for extraction. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in dark glass. The collected extracts were then stored in a refrigerator at 5°C for further experiments.

Antifungal activity of the plant extracts

One ml of extract was incorporated into potato dextrose agar (PDA) medium just before pouring in sterilized Petri dishes. Petri dishes were centrally inoculated with 1 cm fungal disc and incubated at 25±2°C for 5 days. PDA plates without culture filtrate inoculated with pathogen disc served as control. Three replicates were maintained for each treatment. The radial growth of the colony was measure after 5 days and inhibition % of mycelial growth was calculated over the control using the following formula: (Fnug-Tomc *et al*.,1995).

Per cent inhibition (I %) = (C-T/C) x 100

Where, C- mycelial growth of pathogen in control,

T- mycelial growth of pathogen in the treatment

Statistical analysis:

Data were statistically analyzed as a factorial in Randomized Complete block design (16 isolate x 5 Cotton cultivars) with three replicates according to Gomez and Gomez (1984). Least significant differences values (L.S.D.) at 0.05 level of probability were used to compare the differences between treatment means.

RESULTS AND DISCUSSION

Isolation of *R. solani*

A total of 15 isolates of *R. solani* were obtained from cotton planting areas of Egypt. Six isolates were obtained from Alexandria (Giza 45, Giza 87, Giza 92, Giza 93, Giza 94 and Giza 95), one isolate from Algharbia (Tanta-kotana), one isolate from Benysuif (Sids Giza 95). Three isolates obtained from

Elbeheira (two from Abouhomos and one from Damenhour), two isolates from Dkahleya, one from Kafrelshekh and one isolate from Menofyea.

Pathogenicity test of *R. solani*

Pathogenicity of Fifteen isolates of *R. solani* from seven different localities in Egypt was tested on five cotton cultivars (Giza 80, 87, 93, 90 and 92) Table (1 & 2). Pre- and post-damping off was calculated for the 15 isolates. For the pre-damping-off data in Table 1 showed that the isolate from Kafer El-Sheikh was the highest percentage (98%) forward by Monufya (94%) then Damanhur by 82%. While the lowest values recorded to Giza 92 by 16.66%, Giza 45 and 94 by average was almost 17%. High significant variation was observed between the current cotton cultivars as present in Table 1. the data indicated that Giza 80 was more tolerant and recorded 23.13% comparing with the susceptibly one Giza 93 which showed 60.65% and both Giza 87 and 92 showed moderate tolerant to fungal. Concerning to post damping-off the data showed that Abohomos 1 recorded the high value was 10.96% forward by Giza 92, 93 and Dakhlyia 2 by average was near 9% (Table 1). While, the lowest values recorded to Kafer El-shikh and Giza 45 in average was 2.35%. Giza 92 cotton cultivar showed high susceptibility to fungal and recorded 7.08% forward by Giza 87 and 90 in average was 6.4%. Giza 93 showed the most tolerant to fungal and showed 3.47%. From these data, we found that in Table (2) and Figure (1) that Kafer El-Shikh was the virulent isolate and achieved 100% on the other hand Giza 45 showed the avirulent isolate by 20%. Cotton Giza 80 was the tolerant cultivar (28.33%) and Giza 93 was the susceptible cultivar by 64.12%. Similar results were reported by Monga and Sheo-Raj (1994), Aqil and Batson (1999) and Asran (2001). This result is consistent with the findings of Rush *et al*. (1994), El-Akkad (1997) and El-Samawaty (1999), who reported similar trends on Egyptian cottons.

Table (1). Pre- and post-emergence damping-off on cotton cultivars inoculated with *R. solani* isolates

ISOLATES	PRE %					MEAN	POST%					MEAN
	G80	G87	G93	G90	G92		G80	G87	G93	G90	G92	
Beni Suef	20.00	77.77	92.59	88.88	86.66	**73.18^{b}**	10.00	11.11	0.00	11.11	3.33	**7.11^{abc}**
Abohomos 1	3.33	16.66	44.44	48.14	56.66	**33.85^{cd}**	6.66	16.66	3.70	11.11	16.66	**10.96^{a}**
Damanhor	96.66	61.11	74.07	96.29	83.33	**82.30^{b}**	0.00	11.11	3.70	0.00	10.00	**4.96^{abcd}**
Abohomos 2	33.33	88.88	92.59	92.59	90.00	**79.48^{b}**	16.66	0.00	3.70	7.40	0.00	**5.55^{abcd}**
Dakhlyia 1	46.66	88.88	96.29	92.59	86.66	**82.22^{b}**	10.00	0.00	0.00	7.40	3.33	**4.14^{bcd}**
Dakhlyia 2	0.00	55.55	51.85	59.25	40.00	**41.33^{c}**	0.00	16.66	11.11	7.40	13.33	**9.70^{ab}**
Gharbia	0.00	11.11	48.14	18.51	16.66	**18.88^{e}**	6.66	5.55	0.00	7.40	3.33	**4.59^{bcd}**
Kafer El-Shikh	100.00	10000	100.00	100.00	90.00	**98.10^{a}**	0.00	0.00	0.00	0.00	10.00	**2.00^{cd}**
Monofia	70.00	100.00	100.00	100.00	100.00	**94.10^{a}**	20.00	0.00	0.00	0.00	0.00	**4.00^{bcd}**
G45	0.00	5.55	44.44	18.51	20.00	**17.70^{e}**	0.00	0.00	0.00	3.70	10.00	**2.74^{cd}**
G87	0.00	5.55	44.44	25.92	33.33	**21.85^{e}**	3.33	5.55	3.70	3.70	13.33	**5.92^{abcd}**
G92	0.00	11.11	40.74	14.81	16.66	**16.66^{e}**	3.33	16.66	14.81	3.70	10.00	**9.70^{ab}**
G93	0.00	11.11	37.03	18.51	23.33	**18.00^{e}**	0.00	16.66	7.40	11.11	10.00	**9.03^{ab}**
G94	0.00	11.11	40.74	37.03	0.00	**17.77^{e}**	3.33	5.55	3.70	14.81	6.66	**6.81^{abc}**
G95	0.00	27.77	51.85	22.22	23.33	**25.03^{de}**	3.33	0.00	3.70	11.11	3.33	**4.29^{bcd}**
CONTROL	0.00	66.66	11.11	11.11	0.00	**17.78^{e}**	0.00	0.00	0.00	0.00	0.00	**0.00^{d}**
Average	**23.13^{d}**	**46.18^{c}**	**60.65^{a}**	**52.78^{b}**	**47.92^{bc}**		**5.21^{ab}**	**6.60^{ab}**	**3.47^{b}**	**6.25^{ab}**	**7.08^{a}**	

*LSD0.05 for cultivar×isolate interaction= 24.25

*LSD0.05 for cultivar×isolate interaction=13.51

Table (2) Infection percentage of cotton cultivars inoculated with *R. solani* isolates

Isolates \ Cultivars	Infection percentage (%)					MEAN
	G80	G87	G93	G90	G92	
Beni Suef	30.00	88.88	92.59	100.00	90.00	**80.29^{c}**
Abohomos 1	10.00	33.33	48.14	59.25	73.33	**44.81^{d}**
Damanhor	96.66	72.22	77.77	96.29	93.33	**87.25^{bc}**
Abohomos 2	50.00	88.88	96.29	100.00	90.00	**85.04^{c}**
Dakhlyia 1	56.66	88.88	96.29	100.00	90.00	**86.37^{c}**
Dakhlyia 2	0.00	72.22	62.96	66.66	53.33	**51.03^{d}**
Gharbia	6.66	16.66	48.14	25.92	20.00	**23.48^{ef}**
Kafer El-Shikh	100.00	100.00	100.00	100.00	100.00	**100.00^{a}**
Monofia	90.00	100.00	100.00	100.00	100.00	**98.00^{ab}**
G45	0.00	5.55	44.44	22.22	30.00	**20.44^{ef}**
G87	3.33	11.11	48.14	29.62	46.66	**27.77^{ef}**
G92	3.33	27.77	55.55	18.51	26.66	**26.37^{ef}**
G93	0.00	27.77	44.44	29.62	33.33	**27.03^{ef}**
G94	3.33	16.66	44.44	51.85	6.66	**24.59^{ef}**
G95	3.33	27.77	55.55	33.33	26.66	**29.33^{e}**
Control	0.00	66.66	11.11	11.11	0.00	**17.77^{f}**
Average	**28.33^{d}**	**52.78^{c}**	**64.12^{a}**	**59.03^{ab}**	**55.00^{cb}**	

*LSD0.05 for cultivar×isolate interaction= 24.88

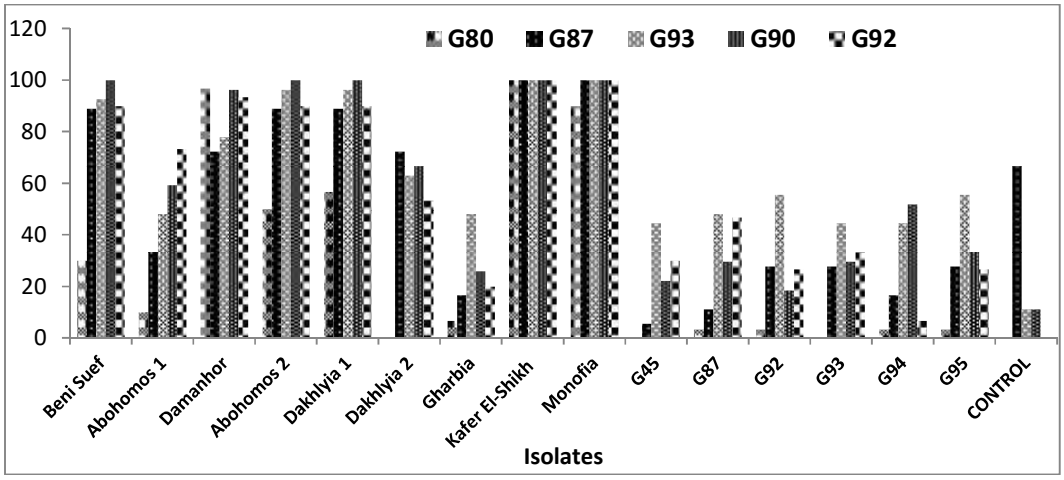

Figure (1). Pathogenicity test of *R. solani* isolates on cotton cultivars

Effect of different plant extracts on *R. solani* growth and inhibition

Data in Figures (2-5) showed the effect of six different plant extracts i.e. pomegranate, banana, wheat bran, liquorice, cinnamon and turmeric on *R. solani* growth and inhibition comparing with control in two isolates namely Kafer El-Shikh and Giza 45. Control showed the same value under all the plant extract (9 cm of *R. solani* growth). while the boiling water method recorded different values ranged from 2 – 9 cm in average 7.55 cm. No significant variations were observed for the pomegranate, banana, wheat bran plant

extracts. On the other hand, liquorice, cinnamon and turmeric showed different inhibition values under boiling water for the fungal growth (Figures 2-5). The combination between alcohol and distilled water showed the same value that was 1 cm. Turmeric showed the lowest fungal growth that was 3 cm in Kafer El-shikh and 2 cm in Giza 45. This revealed that plant extracts could be useful tools to control *R. solani* due to the active components in such natural compounds as reprehensive in turmeric. The growth inhibition of plant extracts of pomegranate, banana, wheat bran, liquorice, cinnamon and turmeric against *R. solani* in two isolates, Kafer El-Shikh and Giza 45 were recorded in Figures 2-5. The current study is in agreement with Sunita and Sharma (2014). that controlled *R. solani* using different plant extracts (botanicals), biofumigant under *in vitro* and field conditions. Out of 11 botanicals including two commercial formulations of neem (neemgold, neemazil) tested at 5,10,20,30,40% and 1,2,3,4,5% concentrations revealed that seed extracts of *Melia azedarach* and leaf extract of *Adhatoda vasica* showed maximum inhibition in mycelial growth within the range of 44.38 to 44.25% followed by *Murraya koenigii* (37.77%) and Tagetes erecta (37.62%). The results indicated that higher concentration resulted in more inhibition of the *R. solani*, above 50 % inhibition was recorded at concentration of 40%, maximum being in neemgold (60.53%) compared to 5% or 10%. This finding is in agreement with that of Beg and Ahmad (2002). The *in vitro* efficacy of cinnamon was reported against different pathogens by Ozcan and Chalchat (2006), Sukatta *et al*. (2008) and Sitara *et al*. (2008). The antifungal effect of plant extracts is related to the chemical composition. The main constituent of cinnamon oil is cinnamaldehyde, which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity (Wang *et al*., 2005) and it may be a potential lead compound for the development of antifungal drugs through the control β-(1,3)-glucan and chitin synthesis in fungi (Bang *et al.,* 2000).

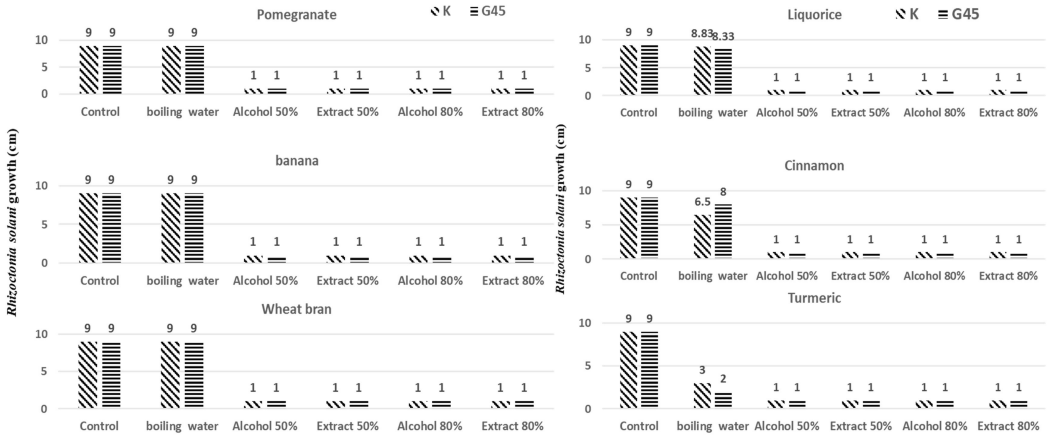

Figure (2). Effects of pomegranate, Banana, Wheat bean Liquorice, Cinnamon and Turmeric with different alcohol concentration on *R. solani* growth

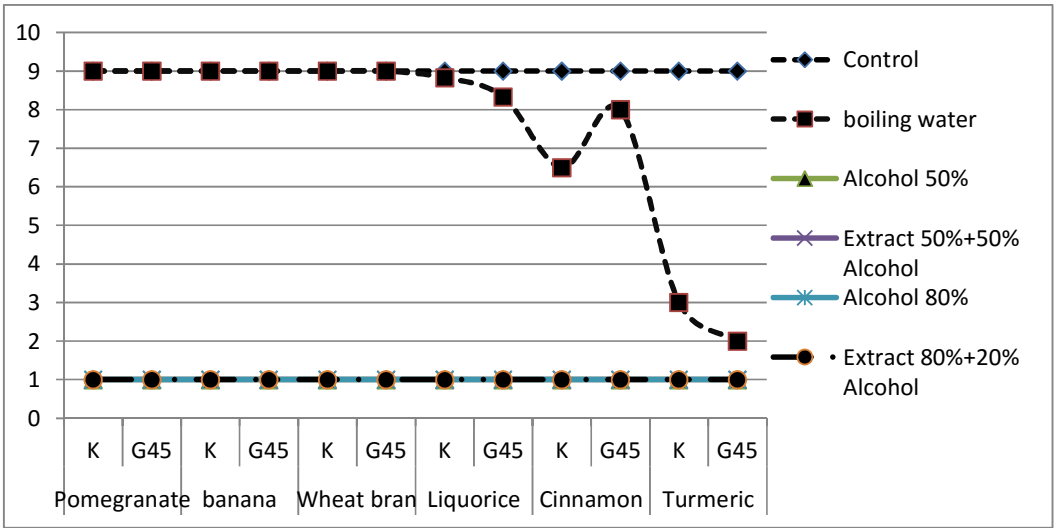

Figure (3). Effect of different plant extracts on radial growth of *R. solani*.
K= isolate from Kafer El-Shikh governorate
G45= isolate from cultivar G45 from Alexandria governorate

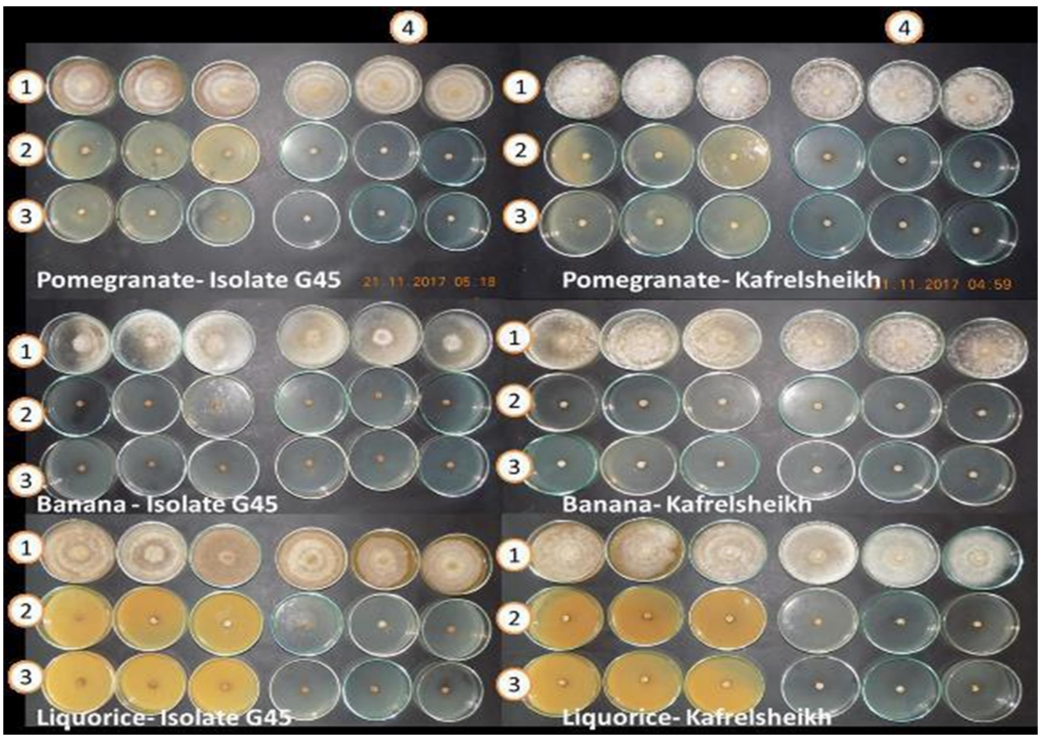

Figure (4). Effects of pomegranate, banana, liquorice on R. solani growth and with control in two isolates Kafer El-Shikh and Giza 45 (1- boiling water, 2- 50% alcohol, 3- 80% alcohol, 4- Control)

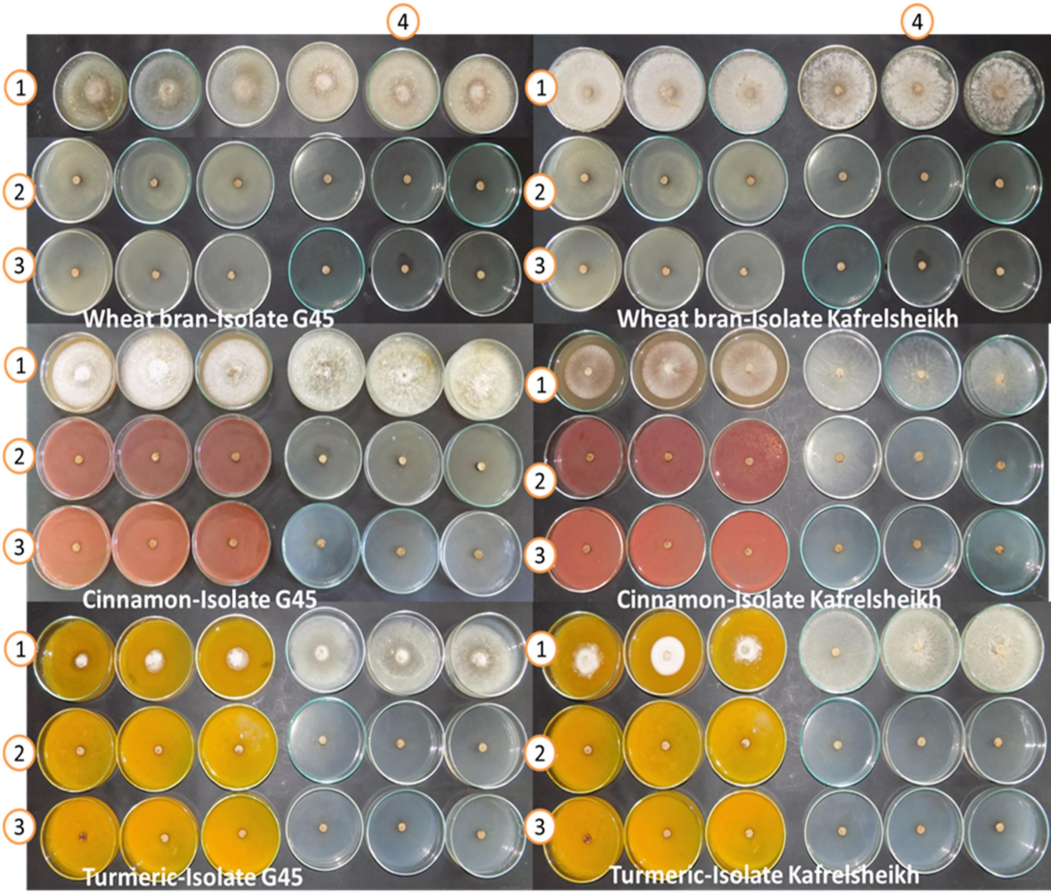

Figure (5). Effects of wheat bran, cinnamon and turmeric on R. solani growth and with control in two isolates Kafer El-Shikh and Giza 45 (1- boiling water, 2- 50% alcohol, 3- 80% alcohol, 4- Control)

CONCLUSION

R. solani Kuhn is the most important fungal disease that caused pre- and post- emergence damping-off, sore shin and root rot of cotton seedlings. Data showed that the isolate from Kafer El-Shikh was the highest one followed by Monufya then Damanhor while, Giza 92 was the lowest isolate. For cotton cultivars data indicated that Giza 80 was the most tolerant cultivar comparing with the susceptibly cultivar Giza 93 and both Giza 87 and 92 showed moderate tolerant to fungal. For plant extract the Turmeric extract showed the lowest fungal growth in Kafer El-shikh and Giza 45 isolates and it can be recommend as bio control against the *R. solani*.

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الملخص العربي

فاعلية بعض المستخلصات النباتية ضد فطر الريزوكتونيا سولانى المسبب لمرض موت البادرات في نبات القطن

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يعتبر القطن واحداً من اهم المحاصيل الاستراتيجية على مستوى العالم وبالاخص فى جمهورية مصر العربية. ويعتر فطر الريزوكتونيا سولانى من اهم الفطريات المسببة لموت البادرات فى مراحل النمو المختلفة بالاخص فى مرحلة نمو البادرات. وعلية أجريت هذة الدراسة بكلية الزراعة سابا باشا فى الفترة من ٢٠١٦ حتى ٢٠١٨ باستخدام خمسة عشر عزلة من مناطق مختلفة من مصر هى محافظات: الإسكندرية ، الغربية ، بني سويف (سدس– الجيزة ٩٥) ، البحيرة ، الدقهلية ، كفر الشيخ (قلين) والمنوفية (بئر السبع). اظهرت النتائج ان العزلة من محافظة كفر الشيخ كانت الاكثر شدة مرضية بنسبة ٩٨% تبعها عزلة محافظة المنوفية بنسبة ٩٤% ثم عزلة البحيرة بحوالى ٨٢%. اظهرت العزلة من صنف قطن جيزة ٩٢ الاقل قيمة وصلت الى ١٦.٦٦% واعطت العزلة جيزة ٤٥ و ٩٤ متوسط قدرة ١٧% وكانت الاقل شراسة. اظهرت النتائج ان الصنف جيزة ٨٠ كان الاكثر تحملا للاصابة بنسبة ٢٣.١٣% مقارنة بالصنف الحساس جيزة ٩٣ (٦٠.٦٥%) واظهر كلا من الصنف جيزة ٨٧ و ٩٢ مقاومة متوسطة لهذا الفطر. اظهرت النتائج ايضا ان مستخلص الكركم اكثر فاعلية ضد فطر الريزوكتونيا سولانى مقارنة بباقى المستخلصات المستخدمة فى الدراسة.