

# Influence of Certain Animal Manures on Incidence of Stem Canker and Black Scurf Disease on Potato

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**Abstract:** *Rhizoctonia solani* (Kuhn) is the causal pathogen of stem canker and black scurf disease on potato. Under open greenhouse conditions two isolates of *R. solani* (AG-3) were used to infect Nicola potato cultivar and caused typical symptoms of stem canker and black scurf disease with different disease severities ranged from strong to weak. *In vitro* Addition of chickens, pigeons and cows manure extracts to the media at different concentrations 0-50% (vol/vol) reduced the growth of the tested isolates of the pathogen. The highest reduction of mycelial growth of the pathogen isolates was obtained when pigeons manure extract was added to the growing media at a concentration of 50 % (vol/vol) followed by another concentrations. Under open greenhouse conditions during two growing seasons 2010 and 2011 addition of manures to the soil at 0.5 and 1% weight of the soil before sowing significantly decreased incidence of stem canker and black scurf disease. Generally, cow manure showed the highest effect on controlling the disease followed by pigeons and chickens. On the other hand, concentration 1 % of all manures was more effective on the reducing of disease incidence than the concentration 0.5 %. On the other hand, cow manure was more effective in increasing the eyes germination followed by pigeons and chicken manure and decreased the dead of sprouts, stem canker and black scurf. Treatment with all the kinds of tested manures increased eyes germination of tubers and reduced sclerotia formation on the surface of tubers and hence disease incidence.

**Keywords:** *Rhizoctonia solani*, Potato, Stem canker, Black scurf, animal manures.

## 1. INTRODUCTION

Potato is one of the most important vegetable crops in Egypt and worldwide. *Rhizoctonia* stem canker and black scurf diseases of potato are economically important in many parts of the world [1]. The pathogen is widespread in all potato growing countries [1-3]. The presence of black scurf on seed and table potato tubers reduces tuber quality and marketability. Black scurf causes significant economic losses in potatoes annually [4, 5]. In the temperate zones, yield reduction was estimated to reach 30-40 % [6] and 19.2-34 % in the tropical and subtropical regions [4, 7]. The typical disease symptoms include death of pre-emerging sprouts, cankers on the underground stem parts and stolons, diminished root system, and sclerotia formation on progeny tubers. Disease severity is not always associated with yield reduction. However, formation of tuber-borne sclerotia downgrades tuber quality [8] with the development of malformed tubers and an alternation in target size and number of tubers [2, 7]. Management of *R. solani* is difficult due to its soil borne nature. The fungus is present in most of the soils and cultivated areas and once it is established in a field, it remains there indefinitely [9]. Chicken manure compost gave better control of *Phytophthora cinnamomi* root-rot on *Lupinus albus* than cow, horse,

or sheep manures. Low incidence of seedling death in chicken manure compost-amended potting mixes correlated with low population of *P. cinnamomi*, high levels of microbial activity in particular the activity of endospore-forming bacteria [10]. Tsror *et al.*, [11] showed that cattle manure compost amendment applied in furrow could reduce black scurf incidence in organically grown potatoes. Although treatment significantly reduced disease incidence and severity, total yield was unaffected. Ekilas, Hamid *et al.*, [12] found that cow manure treatment recorded the lowest *Rhizoctonia* disease incidence. Also, cow manure amendment to soil reduces sclerotia formation in yielded tubers by 17.04–21.48 %. The tuber yield was also improved more than twice as much as using the soil amendment with cow manure.

The present work was planned to study the efficiency of certain animal manures (chicken, pigeons and cow) in controlling potato stem canker and black scurf disease under Egyptian conditions.

## 2. MATERIALS AND METHODS

### 2.1. Effect of Animal Manures Extract on Mycelial Growth of *Rhizoctonia solani* In Vitro

The manures (chicken, pigeons and cow) extracts were prepared according to the seepages procedures of Brinton *et al.*, [13], Elad and Shtienberge [14] and El-Masry *et al.*, [15]. Composted manures were mixed with tap water (1:5 vol/vol) in large containers. The

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suspension was incubated for 6 days at 15 to 20 °C and daily stirred 5 to 10 minutes. After extraction, the mixtures were filtrated through 3 layers of cheesecloth and centrifuged for 60 minutes at 3000 rpm. The filtrates were then sterilized by Seitz filter and stored at 4°C for further studies. Extracts were added to PDA medium at concentrations 5, 10, 20, 30, 40 and 50% vol/vol, and mixed thoroughly before solidification at 50-60 °C and poured in the plates. Petri dishes were inoculated with equal discs (5mm) of the pathogen isolates and were placed in the center of each plate. Petri dishes containing sterilized PDA medium only and inoculated with the fungal isolates were used as control. Four replicates were used for each treatment and all treatments were incubated at 25°C. Isolates No.1 and 2 of *R. solani* (AG-3) which varied in their capability to induce the disease from weak to severe were used in this study. Linear growth of the tested isolates were recorded when the control plates were completely covered by the fungal mycelium. Different concentrations from extracts composted manures were tried until complete inhibition of the fungal growth on the medium was achieved. The inhibition % of the fungal growth was determined after incubation at 25°C for 2-4 days according to the tested isolates of the pathogen. For each isolate, the inhibition percent of each compost extract was determined when the radial growth in the control colonies reached the edge of the plates. Percentage of growth inhibition was calculated according to Kuckuk and Kivanc [16].

$$\text{Growth inhibition Percentage} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

## 2.2. Effect of Animal Manures in Controlling Potato Stem Canker and Black Scurf Disease Under Open Greenhouse Conditions

The animal manures (chicken, pigeons and cow) obtained from El-Beheira Governorate and two isolates of the pathogen (No. 1 and No. 2) were employed in the present studies. The study was conducted during the seasons 2010 and 2011 under open greenhouse conditions of the Faculty of Agriculture, Al-Azhar University, Assiut Branch. Animal manures at the rate 0.5 and 1 % (5 and 10 gm /kg soil) of each of the tested manure [17] were added to the sterilized Clay-sand soil two weeks before sowing and mixed thoroughly then irrigated. Inoculum of each isolate of pathogen was added to the soil in pots at the rate of 1 % (w/w) one week before sowing. Tuber seeds of potato Nicola cultivar were sown in pots containing infested soil with the pathogen isolates No. 1 and 2. Four pots from each treatment were used as replicates. Pots containing

infested soil with the tested isolates of pathogen were sown with tuber seeds and that contain non-infested soil and sown with tuber seeds were served as control. Percentage of eyes germination and death of emerging sprouts were recorded 21 and 45 days after sowing, respectively, while percentage of stem canker and black scurf was recorded when plants showed signs of maturity at about three months from sowing. Percentage of eyes germination inhibition was calculated as mentioned by Farah Naz *et al.*, [18]:

$$\text{Eyes germination (E G) \%} = \frac{\text{number of eyes germinated in each treatment}}{\text{total number of eyes}} \times 100$$

Eyes germination inhibition % = 100- E G %.

$$\text{Sprouts killed \%} = \frac{\text{number of sprouts killed in each treatment}}{\text{total number of sprouts}} \times 100$$

### Stem Canker Index

Stem canker incidence and severity expressed as stem canker index (SCI). Severity was assessed on 0-4 visual disease rating scale as described by Carling and Leiner [19] using the following formula.

$$\text{Stem canker \%} = \frac{\text{number of stems in each rating} \times \text{rating}}{\text{total number of stems}} \times 100$$

Where:

0 = no damage, no lesions.

1 = minor damage, one to several lesions less than 5 mm long.

2 = intermediate damage, lesions longer than 5 mm, girdling of some tissue.

3 = major damage, large lesions, girdling and death of most tissue.

4 = all tissue dead.

### Black Scurf Disease Index

Black scurf disease incidence and severity was expressed as black scurf disease index (BSDI) and was calculated according to Farah Naz *et al.*, [18].

$$\text{Black Scurf Disease \%} = \frac{0(n_1) + 1(n_2) + 2(n_3) + 3(n_4) + 4(n_5) + 5(n_6)}{N \text{ (total number of tubers)}} \times 100$$

Where, n1 = number of tubers in 0 rating; n2 = number of tubers in 1 rating; n3 = number of tubers in 2 rating; n4 = number of tubers in 3 rating; n5 = number of tubers in 4 rating; n6 = number of tubers in 5 rating.

Where, 0 = no symptoms on potato tubers; 1 = less than 1 % tuber area affected; 2 = 1-10 % tuber area affected; 3 =11-20 % tuber area affected; 4 =21-51 % tuber area affected; 5 = 51 % or more tuber area affected.

### 2.3. Statistical Analysis

The obtained data were subjected to statistical analysis using MSTATC computer program (Michigan Statistical Program Version C). Least significant difference (L. S. D.,  $p=0.05$ ) for comparison between means of treatments was used as mentioned by Gomez and Gomez [20].

## 3. RESULTS

### 3.1. *In Vitro*, Inhibitory Effect of Animal Manures Extracts on *Rhizoctonia solani* Growth

Effect of manure extracts (chicken, pigeons and cow) on mycelial growth of *R. solani* AG-3 was evaluated *in vitro* on PDA medium. Data presented in Table (1) indicate that the growth of *R. solani* isolates was influenced significantly by the addition of tested manures extract to the medium when compared to the control. In general, the addition of the tested manure extracts to the medium reduced the growth of the tested isolates of the pathogen. The highest reduction of mycelial growth of pathogen isolates was obtained when pigeons manure extract was added to the growing media, followed by chicken manure extract. However, cow manure extract caused the lowest reduction of mycelial growth of the pathogen isolates.

In fact, the reduction of mycelial growth of pathogen isolates increased gradually by increasing the concentration of manures extract.

Data also indicated that the highest inhibition % in fungal growth occurred to *R. solani* isolate No. 1 was by adding extract of pigeons manure at concentration 50%, while the least inhibition % occurred in *R. solani* isolate No. 2 by addition extract of cow manure at concentration 5 %.

### 3.2. Effect of Some Animal Manures (Chicken, Pigeons and Cow) on Controlling *Rhizoctonia* Disease of Potato Under Open Greenhouse Conditions

The effect of treatment with manures at concentrations 0.5 and 1 % on *Rhizoctonia* disease of potato was studied during two seasons 2010 and 2011 under open greenhouse conditions. Results of this study are present in Tables (2 and 3) and Figure (1) indicate that addition of manures (chicken, pigeons and cow) to the soil before sowing increased significantly eyes germination % in both seasons compared to the control. Generally, cow manure gave the highest eyes germination percentage at concentration 1%, followed by pigeons manure. While, chicken manure gave the lowest effect. At the same time, the seedlings were very strong when compared to the control.

Data also showed that all tested manures were effective in reducing dead sprouts percentage, increased survival plants and improved plant growth characters compared to the control. Generally, cow

**Table 1: Inhibitory Effect of Animal Manure Extracts on *Rhizoctonia solani* Growth *In vitro***

Conc.	Treatments	Growth inhibition %								
		Chicken manure			Pigeons manure			Cow manure		
		Iso.1	Iso.2	Mean	Iso.1	Iso.2	Mean	Iso.1	Iso.2	Mean
0(control)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	12.50*	10.28	11.39	23.61	22.50	23.06	4.44	3.61	4.03	
10	20.56	16.67	18.61	28.34	26.95	27.65	14.16	12.22	13.19	
20	23.05	21.39	22.22	36.67	34.16	35.42	20.83	18.89	19.86	
30	27.36	25.97	26.67	43.86	41.11	42.49	25.56	24.44	25.00	
40	33.61	30.83	32.24	51.11	50.00	50.56	30.00	29.72	29.86	
50	38.61	38.06	38.34	54.72	54.16	54.44	34.72	33.89	34.31	
Mean	22.25	20.46	-	34.05	32.70	-	18.53	17.54	-	
L.S.D. at 5% for:										
Concentrations =A	1.26			0.82			1.07			
Isolates =B	0.68			0.44			0.57			
Interaction =A×B	1.79			1.15			1.51			

\*= Pathogen growth reduction %.

**Table 2: Effect of Some Animal Manures on Controlling Rhizoctonia Disease of Potato Under Open Greenhouse Conditions at Season 2010**

Treatments		Ungermination eyes %		Dead sprouts%		Stem canker %		Sclerotia formation %	
Manures	Conc.	Iso.1	Iso.2	Iso.1	Iso.2	Iso.1	Iso.2	Iso.1	Iso.2
Chicken	0.5 %	37.50	45.83	14.58	22.92	26.90	36.00	29.37	58.45
	1 %	29.17	41.67	8.33	14.58	22.17	27.94	18.00	33.70
Pigeons	0.5 %	29.17	41.67	13.33	19.58	20.80	25.36	20.71	31.40
	1 %	16.67	29.17	9.17	12.50	14.65	23.50	17.50	20.84
Cow	0.5 %	20.83	33.34	12.50	16.67	16.36	25.05	12.52	15.75
	1 %	12.50	20.83	4.17	10.42	13.80	21.21	10.60	12.45
Cont. (untreated)		66.67	79.17	33.33	50.00	65.30	81.38	70.00	85.06
$\bar{X}$		30.36	41.67	13.63	20.95	25.71	34.35	25.53	36.81
L.S.D. at 5% for:									
Treatments = (A)		12.02		18.02		1.10		2.10	
Concentrations = (B)		8.50		12.74		0.78		1.48	
Isolates = (C)		8.01		14.32		1.00		0.97	
Interaction = A×B×C		22.66		40.51		2.83		2.75	

**Table 3: Effect of Some Animal Manures on Controlling Rhizoctonia Disease of Potato Under Open Greenhouse Conditions at Season 2011**

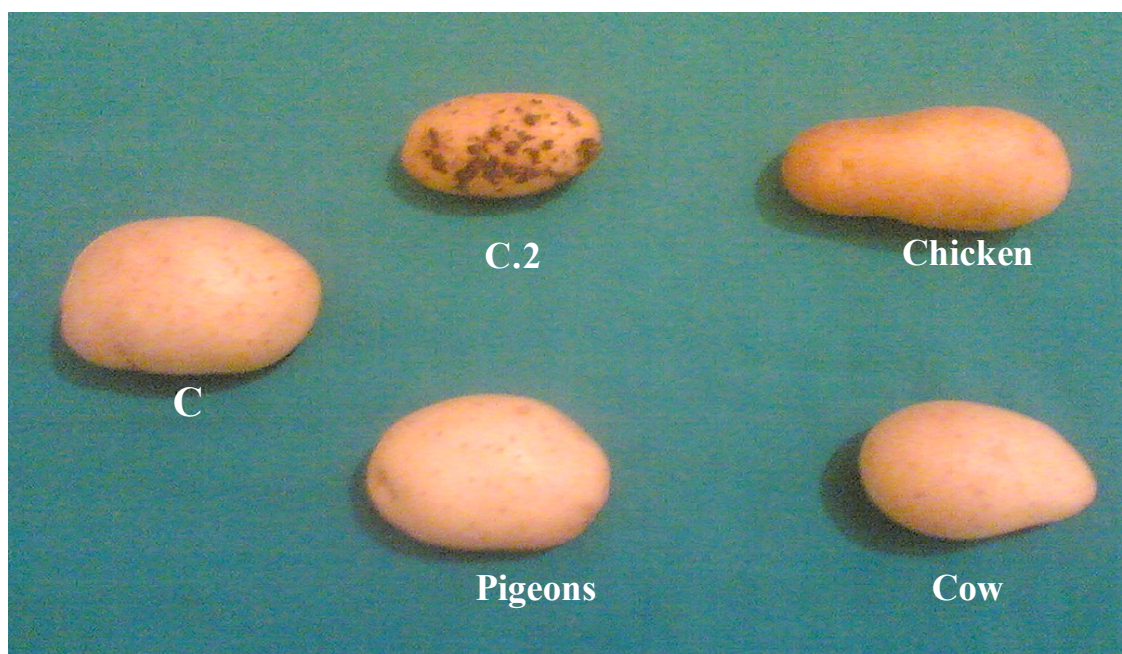
Treatments		Ungermination eyes %		Dead sprouts%		Stem canker %		Sclerotia formation %	
Manures	Conc.	Iso.1	Iso.2	Iso.1	Iso.2	Iso.1	Iso.2	Iso.1	Iso.2
Chicken	0.5 %	29.17	41.67	16.25	25.00	22.30	31.07	27.65	38.12
	1 %	25.00	37.50	11.25	20.83	19.30	26.60	20.20	32.72
Pigeons	0.5 %	33.34	37.50	13.33	20.83	18.88	25.23	22.27	27.43
	1 %	20.83	33.34	9.17	20.00	16.10	21.68	18.34	24.22
Cow	0.5 %	16.67	29.17	12.50	19.58	15.30	23.60	16.93	23.40
	1 %	8.34	25.00	5.00	16.25	14.20	20.59	13.81	20.31
Cont. (untreated)		62.42	70.84	39.58	50.00	61.13	69.00	60.35	79.30
$\bar{X}$		27.97	39.29	15.30	24.64	23.89	31.11	25.65	35.07
L.S.D. at 5% for:									
Treatments = (A)		14.22		17.62		1.18		1.83	
Concentrations = (B)		10.06		12.46		0.84		1.29	
Isolates = (C)		9.89		10.73		0.93		1.29	
Interaction = A×B×C		27.98		30.34		2.63		3.66	

manure caused the highest effect on the disease incidence, followed by pigeons manure. While, chicken manure gave the lowest effect in controlling sprouts death disease. Moreover, the sprouts were very strong when compared to the control.

Results also indicate that the addition of manures (chicken, pigeons and cow) to the soil decreased significantly stem canker and black scurf disease in both seasons compared to the control. They also indicated that the tested manures treatments were

effective in reducing stem canker and black scurf disease. Generally, cow manure caused the highest effect on the disease incidence and gave the best control of the disease with isolate No.1. Concentration 1 % of all tested manures was the more effective on Rhizoctonia disease with all parameters than concentration 0.5 %.

Addition of manures to the soil not only resulted in reducing the disease severity but also increased plant height and tuber yield of plant. Treatment with all tested



**Figure 1:** Efficacy of some animal manures on reduction of sclerotia formation on potato tubers.

manures reduced sclerotia formation on the surface of tubers and hence disease incidence.

#### 4. DISCUSSION

Potato is a very popular food source and it is economically the most important vegetable crop grown in the world. Stem canker and black scurf is economically important disease of potato all over the world. This disease causes a reduction in the size of progeny tubers as well as reduction in yield [21].

*In vitro*, the effect of manures extract (chicken, pigeons and cow) on the mycelial growth of *R. solani* isolates was tested on PDA media at different concentrations (0, 5, 10, 20, 30, 40 and 50 % vol/vol). The results showed that all tested extracts exhibited different inhibitory effect on the growth of *R. solani* isolates at different concentrations. In general, all tested manures extracts significantly inhibited the growth of *R. solani* isolates. These results are in conformity with the results obtained by El-Masry *et al.*, [15]; Zhang *et al.*, [22]; and Kerkeni *et al.*, [23]. Data also indicated that pigeons manure extract gave the highest effect on mycelial growth and inhibition percent of the pathogen, followed by chicken manure extract. On the other hand, cow manure extract caused the lowest reduction of mycelial growth of tested isolates of pathogen. Many theories have been developed to explain the mechanisms for which these extracts could suppress the pathogen. This may include production of

antibiotic compounds by beneficial microorganisms [24] and ammonia from poultry manure is very toxic to living organisms [25, 26, 27]. Ras *et al.*, [28] noted that two probable mechanisms of action in terms of phytopathogen suppressiveness were proposed regarding fungal pathogen: (a) mycoparasitism, which involved direct contact between the tested antagonist and the pathogen on the plate whereby a reduction in the pathogen biomass was also observed and subsequent antagonist growth to reduce the pathogen and (b) production of microbial antibiotic, which may spread through the medium, leaving a clear band that separates the antagonist from the pathogen. Chicken manure is lethal to *B. spinulosa*, confirming a previous report [29] which attributed to toxic levels of phosphorus in soils amended with chicken manure compost.

Under open greenhouse conditions, soil amended with these manures (chicken, pigeons and cow) reduced severity either in the form of eyes germination inhibition, dead sprouts, stem canker or in black scurf symptoms. Generally, addition of the manures to the soil has a beneficial effect on the disease. These results are in agreement with those reported by [10, 30, 31, 32, 33, 34, 35]. Data indicated that all manures reduces the disease severity with all parameters. Concentration 1% of all manures was the more effective on the disease reduction than concentration 0.5%. Cow manure was the best in controlling of disease with all parameters (eyes germination

inhibition, sprouts killing, stem canker and black scurf), followed by pigeons manure. While, chicken manure was the lowest effect on the disease. Several mechanisms could explain how the animal manures reduces these soil-borne pathogens. In the first mechanism, beneficial microfloras including compost-derived microorganisms compete for nutrients with plant pathogens in the rhizosphere [36, 37]. The second mechanism involves production of antibiotic compounds by beneficial microorganisms that are effective in controlling various plant pathogens, a process known as antibiosis [24], the third mechanism involves Parasitism and predation of soil inhabiting pathogens by compost inhabiting beneficial microorganisms [37] and the fourth mechanism of biocontrol involves the induction of systemic resistance in plants by microorganisms present in composts or soils [38]. Possible mechanisms for pathogen suppression by compost planting mixes include inhibition of pathogen growth, pathogen survival and reduction of infection (increased host resistance or competition for infection sites) of the host [39]. The manures increased the beneficial microbial population of the soil as *Trichoderma sp.*, *Penicillium sp.* and some isolates of bacteria which may have increased competition for existing nutrients. Beneficial microorganisms play a major role in the suppression of several pathogens. Chicken manure caused a temporary initial increase in soil pH to 8 or higher, which is at the upper limit for *Streptomyces scabies* to cause potato scab. This increase in pH was accompanied by an increase in ammonia levels and the release of this volatile toxic gas may be involved in reducing population levels of *S. scabies* [40]. Also, they mentioned that application of fresh chicken manure was highly effective in reducing the incidence of Verticillium wilt, potato scab and the population of plant parasitic nematodes. Chicken manure application increased soil pH and led to detectable quantities of ammonia and nitrite accumulation in the soil. Ammonia production from poultry manure amended soil has been reported by Tsao and Oster [25] and Schilke-Gartely and Sims [26]. Finally, the obtained data indicated that the application of manures to the soil not only resulted in reducing the disease severity but also increased plant height and tuber yield of plant. Treatment with manures reduced sclerotia formation on the surface of tubers and hence disease incidence.

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Received on 15-02-2012

Accepted on 29-03-2011

Published on 15-04-2012

DOI: <http://dx.doi.org/10.6000/1927-5129.2012.08.01.35>

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