Effectiveness of Insolation on Controlling Peas Seeds Infection with Pathogenic Seed- borne Fungus *Rhizoctonia solani*

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Received 17 May 2017 - Accepted 24 May 2018

ABSTRACT

Rhizoctonia solani is one of the most common pathogenic fungus that is associated with *Pisium sativum* L. and is known to decrease its productivity. This study aimed to compare the effect of insolation with and without covering by polyethylene layers on the infectivity, growth, and germination of artificially infected pea seeds. The results showed that insolation of pea seeds was effective in elimination of this seed-borne fungus especially when covered with double layer of black polyethylene cover. This method of covering and insolation also enhanced the seeds' germination rate. The most effective outcome was obtained by exposure of seeds for a period of 30 days. Thus, insolation and covering with a double-layer of polyethylene could be established as an effective method of biological control against *R. solani* fungus.

Key Words: Fungi, Fungicides, Pathogenic, Polyethylene.

INTRODUCTION

Peas (*Pisium sativum L.*) is counted among one of the most important leguminous crops that is an essential source of nutrients comprising of proteins, carbohydrates, amino acids and vitamins. However, the productivity of this crop is on a decrease because of its exposure to infections especially by fungi. These pathogenic fungi that attack the peas crop are mostly associated with its seeds thus causing a reduction in the production of seeds and overall vitality of crop (Cavaglieri *et al*., 2009). They are capable of attacking the seeds even in its storage place and release an offending odor. An infestation by fungi on these seeds leads to a decrease in their germination ratio thus affecting the productivity of the plant. Mycotoxins released by these fungi contaminate the agricultural products and enter human and animal food chains, thus being a potential threat to the health of living beings (Bryden, 2007).

One of these pathogenic fungus is *Rhizoctonia solani* (*R. solani*), which thrives in both planted and non-planted soil causing diseases in agricultural crops (Olutoyosi *et al*., 2017). In order to control the outbreak of *R. solani* in agricultural products, several methods, both chemical as wellas biological, have been developed and tried by agricultural scientists (Goudjal *et al*., 2014; Donayre *et al*., 2015; Le Cointe *et al*., 2016). The use of chemical methods has declined in the recent years as it affects the environment which is subsequently harmful to other living organisms (human, animals) (Bakkali *et al*., 2008). Thus, the focus has been shifted to developing biological methods of control that would reduce the effective pathogenic power of microorganisms and provide a safer alternative for increasing agricultural production.

One of the cost-effective, easy and safe methods of biological control nowadays is the technique of soil solarization that is run in alignment with the strategy of integrated pest management (IPM) which leads to improved growth and development of plant besides being fatal to pathogens (Bacha *et al*., 2007). However, the success of soil solarization (insolation) depends on the time period of solar radiations and temperature, while its performance can be improved via some modifications in solar technique which includes covering with polyethylene bags so that the heat energy in solar radiations gets trapped inside the seeds. This study aimed to use benefits of environmental conditions such as high temperatures in summer and conduct a number of modifications on some of traditional insulation techniques to get rid

of seed-borne fungi as an alternative, safe, economical and non-polluting method of pathogenic control.

MATERIALS AND METHODS

Two types of peas seeds that were certified and available for agricultural use in Al Jouf region were selected for this study. The difference between these two types of seeds was based on degree of disease tolerance. These were categorized as sensitive (local type) and tolerant (master type).

Preparation of pathogen

A sporangium suspension of *R. solani* was cultivated on potato dextrose agar (PDA) medium at a temperature of 25 °C for a week. A suspension with concentration of about 500000 spore/ ml was prepared and used as infective agent for seeds.

Infection of seeds

Two kg of peas seeds were sterilized by soaking them in a solution of 5% sodium hypochlorite for 5 minutes and then transferred to distilled water to get rid of the effect of sterilization material. This was followed by soaking the seeds in prepared sporangium suspension of *R. solani* for 8 hours which were then transferred to a sterile blotting paper for 48 hours to get rid of excess water.

Examining pathogenicity of *R. solani*

Culture method

Fifty seeds that were previously soaked in the fungal suspension were taken and cultivated in petridishes containing PDA nutrient medium. Five replicates for each dish were used which were incubated at 25 °C for one week. The dishes were then examined to detect the presence of pathogenic fungus. The percentage of germinating seeds and infected seeds was recorded.

Wet tissue method

Another group of seeds were cultivated in petridishes on wet tissue by putting pieces of cotton in between to provide high moisture for seed germination and growth of fungi. Five such replicas were plated and incubated at 25 °C for a week. After the incubation period, plates were examined and the percentage of healthy and infected seeds was recorded **(**Sarhan, 2009).

Insolation and experiment design:

The treated seeds were divided into three groups placed in plastic containers individually: Group 1 was without cover, group 2 was covered with one layer of polyethylene, and group 3 was covered with two layers of polyethylene. Each group had three replicates.

Germination of treated seeds in each group was conducted by planting 200 seeds in sand within the plastic pots of dimensions 20 x 16 cm. These pots were exposed to direct sunlight for sixty days, i.e., from August 10 to October 10, 2015. A thermometer was placed on the surface of pots of each group before covering them and the daily temperature was recorded according to the method of International Seed Testing Association (ISTA) **(**ISTA, 2005). The planted seedlings were counted daily for 8 days to estimate the percentage of germination. Speed of germination was measured using the following equation:

Speed of germination = (number of germinated seeds at first day ÷1) + (number of germinated seeds at second day ÷ 2) + …… + (number of germinated seeds at eighth day ÷ 8).

Root & stem length were measured in centimeter after 10, 20 and 30 days of exposure.

Statistical analysis

Statistical analysis was performed using the SPSS software package (Version 17.0). Significance of data were checked using independent t-test. P-values <0.05 were considered significant.

RESULTS AND DISCUSSION

Exposing seeds to sunlight for a specific period of time has proved to be beneficial for observing the growth of fungus *R. solani*. We tried to elucidate the outcome of this effect in presence or absence of polyethylene coverings on the growing seedlings by studying the rate

of infectivity, speed and percentage of their germination and monitoring the effect on the growth of roots and shoots of these seedlings. Figure 1(a) and (b) represent the impact of *R. solani* seed-borne fungus on germination and infection rate of peas seeds respectively. The local seeds category showed a significant decrease in the rate of germination as compared to master seeds category. This decrease was observed to be more in case of seeds growing on wet tissue than in culture medium. However, reverse was observed for infected seeds. A high ratio of infected seeds were found among master seeds category growing in culture. Local seeds growing on wet paper were also found to have high infection rate. This may be due to high moisture conditions provided by wet tissue method which helps in growth of fungal strands on seeds. Similar results have been reported in other study as well (Abed el Monem *et al*., 2000).

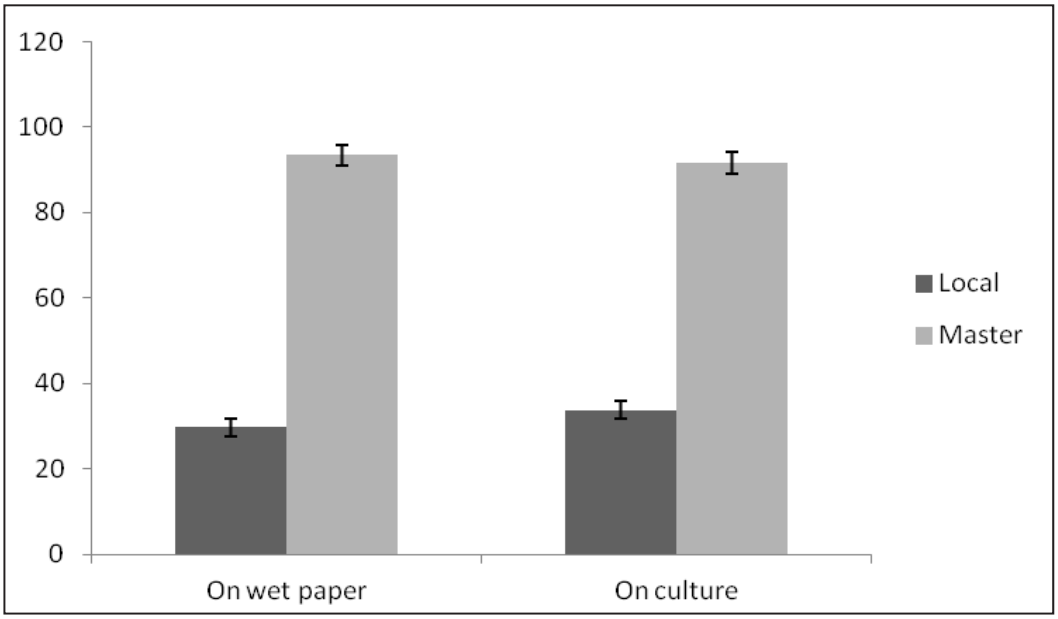

Figure 1 (a). The effect of peas seed-borne *R. solani* on the rate of seed germination. All values are expressed as percent. Data is represented as mean ± SE. *p ≤ 0.05 (Student's t-test)

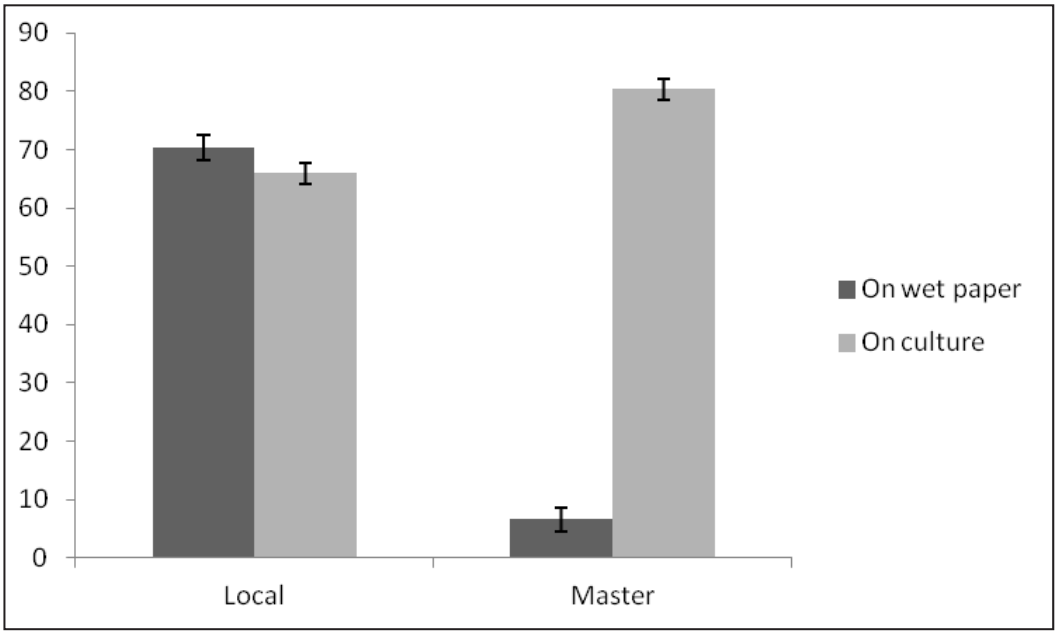

Figure 1 (b). The effect of peas seed-borne *R. solani* on the rate of seed infection. All values are expressed as percent. Data is represented as mean ± SE. *p ≤ 0.05 (Student's t-test)

Percentage of infected seeds

It was observed that the time period of exposure of seeds to sunlight had a significant effect on the percentage of fugal infestation. Exposing the seeds for around 20-30 days significantly decreased the chances of infection of peas seeds by *R. Solani* as compared to control sample that was not exposed to sun (Table 1). Alongwith the duration of exposure to sunlight, covering by layers of polyethylene also played a major role in the percentage of infectivity. In all the cases, a double layered cover was more protective than single layered or leaving the pots uncovered since the percentage of infection was least in seeds that had a double-layered cover in both master and local type of seeds. This finding is in line with some previous studies (Rahmoun B, 2008**).** This was probably because covering with polyethylene traps sun's rays inside the pots and results in increase of temperature. Temperature as high as 40-60 ºC is lethal for growth of fugal cells. High temperature causes changes in metabolism of fungus, denaturing of proteins and accumulation of toxic products which leads to its death (Santiago *et al*, 2014).

Table 1. The effect of insolation and polyethylene covering of peas seeds on infection percentage by *R. solani* fungus, speed and percentage of germination

Exposing periods by day	Master type			Local type		
	Two layers	One layer	Without cover	Two layers	One layer	Without cover
A. Percentage of infection (%)						
0 (Control)	100 ± 0.5	100 ± 1.0	100 ± 0.3	100 ± 1.1	100 ± 1.0	100 ± 0.3
10	98 ± 1.8	100 ± 0.2	100 ± 1.5	100 ± 1.9	98 ± 2.3	96 ± 1.4
20	75.7 ± 2.3	83.2 ± 3.2	90.1 ± 2.9	73 ± 3.1	80.4 ± 2.2	86.7 ± 2.6
30	11.7 ± 1.1*	41.3 ± 1.4*	77.3 ± 2.2*	7.3 ± 1.7*	37.3 ± 1.9*	70.7 ± 3.1*
B. Speed of seed germination						
0 (Control)	16.6 ± 1.2	16.4 ± 2.1	17.3 ± 1.3	27.1 ± 2.5	27.5 ± 1.5	28.6 ± 2.9
10	15.6 ± 2.3	14.7 ± 1.7	14.4 ±1.9	27.7 ± 1.2	22.5 ± 1.7	20.7 ± 1.9
20	16.9 ± 2.1	15.6 ± 1.9	12.9 ± 2.0	20.6 ± 2.3	16.3 ± 2.1*	16.2 ± 1.2*
30	19.6 ± 1.9*	16.1 ± 2.2	9.2 ± 2.1*	17.8 ±1.5*	14.7 ± 2.3*	9 ± 0.6*
C. Percentage of germination (%)						
0 (Control)	65.1 ± 1.9	65.1 ± 2.4	65.1 ± 1.9	97 ± 1.3	96.6 ± 2.6	98 ± 2.5
10	66.3 ± 1.4	65.7 ± 1.1	63.3 ± 1.7	97.6 ± 2.4	87.3 ± 2.8	92.1 ± 1.9
20	66.3 ± 2.2*	66.3 ± 1.5	59.7 ± 2.3	93.7± 1.8	89.4 ± 4.3	95.3 ± 2.4
30	84.7 ± 2.3*	75.3 ± 2.0*	34.3 ± 2.2*	83.7 ± 1.7*	74.3 ± 2.1*	50.6 ± 1.6*

Data is represented as mean ± SE. *$p \leq 0.05$ (Student's t-test)

Speed and percentage of germination

It was observed that there was no significant difference in the speed or percentage of germination of seeds as compared to control when exposed to sun for 10 or 20 days under double or single layers of polyethylene. However, the percentage of germination in master type seeds increased when exposed to sun for a period of 30 days under double layered cover. Both in master and local types of seeds, an exposure of 30 days of sunlight with no polyethylene cover led to decrease in speed and percentage of germination (Table 2).

A few other studies have established solarization to be an effective technique of pest control in seeds that are left for germination and also in growing plants (Pinkerton *et al*., 2000; Triki *et al*., 2001)

Table 2. Effect of insolation and covering on root and shoot length of peas seeds

Exposure period	Local type			Master type		
	Without Cover	One layer	Two layers	Withoutcover	One layer	Two layer
ROOT LENGTH						
0 (Control)	14.1 ± 1.2	14.3 ± 1.4	14.5 ± 1.6	14.9± 1.6	14.7 ± 2.1	14.9 ± 1.7
10	13.2 ± 2.1	10.5 ± 1.6 *	11.3 ± 1.0	12 ± 1.3	9.8 ± 1.5 *	7.6 ± 1.1 *
20	11.6± 1.1 *	11.4 ± 2.3 *	7.7 ± 2.1 *	11.7 ± 1.6	10.1 ± 1.8 *	7 ± 1.3 *
30	15.7 ± 1.5	9.9 ± 1.3 *	8.9 ± 1.5 *	14.4 ± 1.7	9.7 ± 1.6 *	4.2 ± 1.0 *
STEM LENGTH						
0 (Control)	10 ± 2.1	10 ± 1.3	10 ± 1.0	8.9 ± 1.3	8.9 ± 1.3	8.9 ± 1.2
10	9.5 ± 1.7	9.7 ± 1.8	7.6 ± 1.7 *	6.9 ± 1.4v	7 ± 1.2	6.7 ± 1.6
20	9.4 ± 1.8	7.6 ± 1.0 *	7 ± 1.8 *	6.8 ± 1.6 *	6 ± 1.0 *	5.9 ± 1.8 *
30	9.6 ± 1.5	6.8 ± 1.4 *	7.4 ± 1.2 *	7.3 ± 1.7 *	5.6 ± 1.4 *	5.3 ± 1.6 *

Data is represented as mean ± SE. *p ≤ 0.05 (Student's t-test)

Root and shoot length

It was observed that the root and shoot length of peas seedlings got significantly decreased under covering with polyethylene layers on exposure to specific periods of sunlight in both local and master seeds category. However, the lengths of root and stem was not effected when exposed to sun for some period of time without any cover (Table 2). To the best of our knowledge, no such measurements under these experimental conditions have been taken till date, hence this study is the first of its kind.

In conclusion, exposing peas seeds to solar radiation for a period of 30 days with a double layered covering of polyethylene was an effective method of controlling the growth and infectivity of *R. solani*, which also increased the germination rate of seeds. Further experiments need to be conducted with varying exposure periods to sunlight which would establish this method as a productive biological fungal control by producing very high rates of germination.

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فعالية التشميس في مكافحة عدوى بذور البازلاء بالفطريات المسببة للأمراض *Rhizoctonia solani*

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استلام 17 مايو 2017م - قبول 24 مايو 2018م

الملخص

يعد الفطر *Rhizoctonia Solani* من أهم فطريات التربة الممرضة والمصاحبة لبذور البازلاء *Pisium Sativum L* وفي هذه الدراسة تم تنفيذ تجربة لمكافحة فطر *R. Solania* المحمول على بذور البازلاء المعداة صناعيًّا؛ وذلك عن طريق تشميس البذور تحت غطاء من أكياس البولي إيثيلين الأسود لفترات زمنية متفاوتة وبعدد من الأغطية المختلفة.

أظهرت نتائج التحليل الإحصائي أن تشميس بذور البازلاء فعال في القضاء على الفطر المحمول على البذور التي تمت عدواها صناعيًّا، وأن استخدام طبقتين من البولي إيثيلين الأسود كان أكثر فعالية ضد الفطر الممرض. كما أثر التشميس إيجابيًّا في إنبات البذور للصنف (المحلي) المغطاة بالبولي إيثيلين الأسود ، وأوضحت النتائج أن تأثير التشميس لمدة ثلاثين يومًا باستخدام طبقتين من غطاء البولي إيثيلين كان أفضل المعاملات.

ونتيجة لهذه الدراسة تمت التوصية باستخدام تشميس التربة بطبقة مزدوجة من البولي إيثيلين كطريقة آمنة بيئيًّا.

الكلمات المفتاحية: البولي إيثيلين، الفطريات، المبيدات الفطرية، المسببات المرضية.