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A comparison between acid seed delinting and fungicide seed treatment in controlling cotton seedling damping-off disease*

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ABSTRACT: Seed decay (rot) and seedling damping-off caused by *Rhizoctonia solani* are among the most important diseases of cotton which result in the significant yield reduction, application of expensive and harmful chemical fungicides and most importantly contamination of soil and agricultural environment. This study was conducted to investigate the possible efficacy of cotton seed delinting in the reduction of seeds and seedlings susceptibility to the above-mentioned diseases in comparison with the commonly used fungicide. Sulfuric acid which is commonly used for cotton seed delinting at three concentrations (60%, 80% and 98%) was used in the study and two domestic cotton varieties (Sahel and Varamin) was selected for this study. The seeds were subjected to the delinting process at the library using the above-mentioned concentrations following the standard procedure. The effects of seed delinting on seed rot and seedling damping-off diseases were then investigated in a greenhouse experiment where delinted seeds were sown in plastic pots containing cotton field soil pre-inoculated with the inoculum of *Rhizoctonia solan*i in comparison with Carboxin-thiram, the commonly used fungicide for controlling the above-mentioned diseases. The effectiveness of acid seed delinting and fungicide seed treatment in controlling the diseases was evaluated by determining and comparing the number of emerged and survived seedlings in different treatments 15 and 60 days after sowing. Results indicated that seed drlinting with 98% sulfuric acid was the most effective treatment and resulted in a significant reduction in both pre-emergence and post emergence damping-off disease of cotton seedlings in both varieties. Seed delinting with 60 and 80% acid and treating seed with Carboxin-thiram fungicide in some experiments were effective but their effectiveness was much less than 98% acid. The overall results of this study suggest that cotton seed delinting may be an important factor in cotton cultivation and can significantly increase the emergence and number of healthy seedlings.

Key words: Cotton, seed rot, seedling damping-off, *Rhizoctonia solani*, seed delinting, sulfuric acid, carbixin-thiram.

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INTRODUCTION

Cotton is an important cash crop in many countries including Iran. It is primarily the world's major source of fiber and has several other applications including nutritional and medical. The cultivation and production of cotton have great impact on the economy and development of many countries in the world (Heydari *et al*., 2008).

In Iran like many other developing countries, cotton is a major agricultural crop and is cultivated in more than half of the provinces of the country (Ardakani *et al*. 2009; Heydari *et al.,* 2005; Naraghi *et al.* 2007). Plant pests including \insects, weeds and pathogens are among the most important yield redacting factors in cotton fields (Heydari *et al.,* 1998; Heydari *et al.*, 2005; Heydari *et al.,* 2008; Naraghi *et al*. 2007; Watkins 1981). Seedling damping-off disease caused by *Rhizoctonia solani* is one of the most important and damaging factors in Iranian cotton fields (Heydari *et al*., 2005).

Rhizoctonia solani Kuhn is a wide host range plant pathogenic fungus which causes different diseases on many plants and is the major causal agent of pre-and post emergence damping-off disease of cotton seedling in

Iran (Heydari *et al.*, 2005; Khedri *et al* 2014a; Khedri *et al* 2014b; Ogoshi 1987; Omar *et al.,* 2009*;* Watkins 1981). *R. solani* is composed of genetically isolated groups (Adams 1988). The identification and classification of these groups is primarily based on anastomosis behavior (Ogoshi 1987). To date, 14 anastomosis groups (AGs) have been identified for this pathogenic fungus (Carling *et al.,* 2002; El-Saawaty 2008). Some isolates of *R. solani* AG-2-2, AG-4 and AG-5 have been reported to reduce emergence of cotton seedlings (Grosch *et al.,* 2004; Ogoshi 1987; Rush *et al.,* 1994).

Damping- off or mortality disease of cotton seedlings is one of the most common and damaging diseases of this crop around the world including Iran (Heydari *et al,.* 2005; Watkins 1981) . It occurs in two forms of pre- and post emergence of the seedlings and kills the seedlings either before emerging and or afterward (Watkins, 1981). Seed treatment with chemical fungicides such as Carboxin-thiram is the most common method for controlling this disease. In Iran most cotton growers use the linted seeds in the field which may potentially increase the susceptibility of the seedlings to several possible pathogens carried by the lint (Heydari *et al.*, 2005). In addition, the existence of the lint makes the fungicide seed treatment difficult for the growers and reduces its efficiency.

The objective of this study was to compare the effects of acid seed delinting and fungicide seed treatment in the incidence of pre- and post emergence damping-off of cotton seedlings in the greenhouse conditions.

MATERIALS AND METHODS

Cotton seeds

Cotton seeds of two domestic varieties (Sahel and Varamin) which were susceptible to seedling damping-off disease caused by *Rhizoctonia solani* were obtained from Iranian Cotton Research Institute and were stored in the laboratory for using in the study.

Rhizoctonia solani isolate

A virulent isolate of *R. solani* was obtained from microbial collection of soil born fungal research laboratory, Iranian Research Institute of Plant Protection where this study was conducted. The pathogenic fungus was re-cultured on potato dextrose agar (PDA) culture medium and fresh culture was used in the study.

Acid preparation

Sulfuric acid which is commonly used for cotton seed delinting was selected for this study and was obtained from Tehran chemical market. Three concentrations (60, 80 and 98%) were decided to be used in this study to introduce an optimum effective concentration.

Soil preparation

Soil used in this study was collected from cotton fields in different provinces of Iran where the above mentioned cotton varieties are planted. Collected soils were transferred to the experimental greenhouses at Iranian Research Institute of Plant Protection and were pasteurized using a steam heater. Pasteurized field soils were then placed in plastic pot at 4 kg per pot.

R. solani inoculum preparation

For preparation of *R. solani* inoculum, 500 g of autoclaved wheat was placed in a flask and was inoculated with five 1-cm diameter mycelial plugs of a fresh culture of *R. solani* grown on potato dextrose agar (PDA) culture medium. The flask was then incubated for three weeks at 25 C until the fungal mycelia covered the whole grain surface. The contain of the flask was then evacuated, dried out, blended, and used as inoculum in the greenhouse experiment.

Greenhouse experiment

Two greenhouse experiments with a completely randomized (CR) design each with five treatments and four replications were conducted and performed to evaluate the efficacy of acid seed-delinting and fungicide seed treatment in the control of cotton seedling damping-off disease. Delinted seeds and fungicide treated seeds of two domestic cotton varieties (Sahel and Varamin) were sown in plastic pots (10 seeds per pot) filled with four kg of pasteurized field soil pre-inoculated with the inoculum of *R. solani*. Pots were placed in the green house with 12 h photoperiod and were watered as needed. The number of survived cotton seedlings (stand) was determined in each pot 15 and 60 days after sowing to evaluate the pre- and post emergence damping-off disease incidence in different treatment. The efficacy of acid-seed delinting and fungicide-seed treatment was then evaluated by comparing disease incidence in their respective treatments..

Statistical analyses of the data

data obtained in different experiments were subjected to analysis of variance (ANOVA) and means were compared by Duncan Multiple Range Test using CO-STAT statistical soft ware (Cohort, CA, USA). Level of significance was determined at the 5% probability..

RESULTS

Results of the greenhouse studies are presented in tables 1-4.

Table 1 indicates the effects of acid seed delinting and fungicide-seed treatment in controlling pre-emergence damping-off disease of cotton seedlings for Sahel variety. As the Table shows, seed delinting with 98% acid resulted in the highest number of emerged seedlings and was the most effective treatment in reducing the pre-emergence damping-off disease in comparison with other treatment including the commonly used chemical fungicide (Table 1). Seed delinting with 80% acid although was less effective than 98%, but still reduced the disease significantly comparing to the other treatment (Table 1).

Table 2 contains the results of the comparison between acid seed delinting and fungicide seed treatment in controlling pre- and post emergence damping-off on Sahel cotton variety. According to this Table, in this experiment seed delinting with 98% acid was also the most effective treatment and resulted in the highest reduction in the incidence of pre- and post emergence damping-off disease of cotton seedlings (Table 2). In addition to this treatment, seed delinting with 80 and 60% acid and seed treatment with Carboxin-thiram fungicide also significantly reduced the disease incidence in comparison with linted seeds (Table 2).

The results of comparison between acid-seed dleinting and fungicide-seed treatment in controlling pre-emergence damping-off on Varamin cotton variety is presented in Table 3. As the Table indicates, seed delinting with 98% acid resulted in the highest number of the emerged seedling and the least amount of pre-emergence disease. Seed delinting with 80% acid was the next effective treatment and reduced the disease significantly in comparison with control. Fungicide-seed treatment and seed delinting with 60% acid however, were not significantly effective in controlling the pre-emergence damping-off on Varamin variety (Table 3).

Table 4 indicates the effects of acid-seed delinting and fungicide-seed treatment on the incidence of pre- and post emergence damping- off on Varamin cotton variety. In this experiment also seed delinting with 98% acid was the most effective in reducing pre- and post emergence damping-off incidence (Table 4). Seed delinting with 80% and 60% acid and seed treatment with Carboxin-thiram fungicide were the next and all reduced the pre- and post emergence damping-off on Varamin cotton variety significantly compared to the control (Table 4).

Table 1. Effects of acid seed delinting and fungicide seed treatment on Pre-emergence Damping-off disease on Sahel cotton variety

Treatment code	Treatment descriptions	Number of emerged seedlings 15 days after sowing	Pre-emergence damping-off (%)
Cont.	Seeds with lint	6.25 c	37.50
Car-Tir	Seeds with lint treated with carboxin thiram fungicide	6.50 c	35.00
SA (60%)	Delinted seeds with sulfuric acid (66%)	6.75 c	32.50
SA (80%)	Delinted seeds with sulfuric acid (80%)	7.50 b	25.00
SA (98%)	Delinted seeds with sulfuric acid (98%)	9.00 a	10.00

Values in the columns marked with the same letter(s) are not statistically different (P>0.05)

Table 2. Effects of acid seed delinting and fungicide seed treatment on Pre-and Post emergence damping-off disease on Sahel cotton variety

Treatment code	Treatment descriptions	Number of survived seedlings 60 days after sowing	Pre- and post emergence damping-off (%)
Cont.	Seeds with lint	3.25 d	67.50
Car-Tir	Seeds with lint treated with carboxin thiram fungicide	4.75 c	52.50
SA (60%)	Delinted seeds with sulfuric acid (66%)	5.50 bc	45.00
SA (80%)	Delinted seeds with sulfuric acid (80%)	6.50 b	35.00
SA (98%)	Delinted seeds with sulfuric acid (98%)	8.25 a	17.50

Values in the columns marked with the same letter(s) are not statistically different (P>0.05)

Table 3. Effects of acid seed delinting and fungicide seed treatment on Pre-emergence damping-off disease on Varamin cotton variety

Treatment code	Treatment descriptions	Number of emerged seedlings 15 days after sowing	Pre-emergence damping-off (%)
Cont.	Seeds with lint	5.25 c	47.50
Car-Tir	Seeds with lint treated with carboxin thiram fungicide	6.00 c	40.00
SA (60%)	Delinted seeds with sulfuric acid (66%)	6.25 c	37.50
SA (80%)	Delinted seeds with sulfuric acid (80%)	7.25 b	27.50
SA (98%)	Delinted seeds with sulfuric acid (98%)	8.75 a	12.50

Values in the columns marked with the same letter(s) are not statistically different (P>0.05)

Table 4. Effects of acid seed delinting and fungicide seed treatment on Pre-and post emergence damping-off disease on Varamin cotton variety

Treatment code	Treatment descriptions	Number of emerged seedlings 15 days after sowing	Pre-emergence damping-off (%)
Cont.	Seeds with lint	2,50 d	75.00
Car-Tir	Seeds with lint treated with carboxin thiram fungicide	4.00 c	60.00
SA (60%)	Delinted seeds with sulfuric acid (66%)	5.00 bc	50.00
SA (80%)	Delinted seeds with sulfuric acid (80%)	5.75 b	42.50
SA (98%)	Delinted seeds with sulfuric acid (98%)	8.00. a	20.00

Values in the columns marked with the same letter(s) are not statistically different (P>0.05)

DISCUSSION

The overall results of this study show that acid-seed delinting to remove the lint of cotton seeds may be an effective method to reduce the seed fungal transmission and infection particularly *Rhizoctonia solani* which is an important pathogen of cotton during the early season and causes seedling damping-off. Cotton growers particularly in developing countries including Iran mostly use linted seeds for cotton plantation which has two disadvantages. First, the lint can act as a physical barrier for the germination of the seed and emergence of the seedlings. The second disadvantage could be related to the possible transmission of pathogenic micro-organisms including fungi. The above-mentioned limiting factors may result in the significant reduction in the germination and emergence of seedlings and make the farmers to replant and re-cultivate the cotton fields.

Seedling damping-off is one of the most important diseases of cotton in the world including Iran (Heydari *et al.,* 2008; Heydari *et al.,* 2005; Heydari *et al.,* 1998; Khedri *et al.,* 2014 a). This disease usually occurs in two forms of pre-emergence and post emergence. Pre-emergence damping-off, disease causes seed decay or rot which result in the lack of germination and emergence of the seedling (Carling *et al.,* 2002; El-Samawaty *et al.,* 2008). In post emergence form of damping-off which usually occurs up to 4-6 weeks after the emergence, infected cotton

seedlings become necrotic in the root and crown area and finally die and damp on the soil (Carling *et al.,* 2002; El-Samawaty *et al.,* 2008; Khedri *rt al.,* 2014b).

In the present study we worked with *Rhizoctonia solani* because in Iran this fungal pathogen is the major causal agent of the disease and this has been proved in the other studies (Heydari *et al.,* 2005). *R. solani* is a wide host range fungal pathogen with different anastomosis groups which are responsible for the disease on various host plants (Brenneman and Bell, 1995: Adamas, 1988). We in this study used an isolate of *R. solani* belonged to AG-4 anastomosis group because this group is responsible for causing damping-off disease on cotton seedlings which has been shown in the previous research studies (Ogoshi, 1987' Omar *et al.,* 2009).

The most common method for the management of cotton seedling damping-off disease is the use of organic chemical fungicides including Carboxin-thiram. The continuous application of chemical fungicides to control plant diseases has resulted in the appearance of resistant races of the pathogens, negative impacts on non-target organisms and contamination of the agricultural environment (Ardakani *et al.,* 2009; Heydari *et al.,* 2005). Most cotton growers in developing countries use linted seeds in the field which results in poor germination and emergence. On the other hand, the lint can be a potential transmitter of some plant pathogen including *R. solani* as has been shown in the previous studies (Heydari *et al.,* 2005; Khedri *et al.,* 2014a).

The results of our study indicated that seed delinting with sulfuric acid to remove lint of cotton seeds resulted in significant control of seed decay and seedling damping-off diseases. The obtained positive results could be due to the removal of the lint as both physical barrier for germination and also potential transmitter of fungal pathogen. In this study 98% acid was the most effective because its application resulted in the complete lint removal, whereas other concentrations removed the lint partially. The fungicide-seed treatment was not as effective as acid-seed delinting probably because lint is a limiting factor in the treatment process.

The results of the present study may have practical application in the formulation of cotton seedlings damping-off disease management program as an important limiting factors in the cultivation and production of cotton in Ira

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