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**[Short Communication]**

# The Effect of Trifluralin on *Rhizoctonia solani* (isolate AG4), Causal Agent of Soybean Damping off

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## ABSTRACT
Damping off caused by *Rhizoctonia solani* (isolate AG4) constrains growing soybean in the north Iranian province of Golestan. The pathogen was grown on malt agar containing 0, 50, 100 or 200 ppm trifluralin then its vegetative growth and virulence were evaluated. The response of soybean cv. Williams seedlings grown in soil containing 0, 0.5, 1 and 2 µg ai $g^{-1}$ trifluralin was investigated. On malt agar containing trifluralin, the radial growth of the fungal colonies was slower than that on untreated medium. There was no significant difference between these two types of colony in their abaility to cause disease symptoms on soybean seedlings. The incidence of soybean damping off in autoclaved soil treated with trifluralin was significantly higher than that in untreated soil. The results indicate that trifluralin enhanced the susceptibility of soybean radicles to the pathogen.

**Key words:** Damping off,*Rhizoctonia solani*, Soybean ,Trifluralin,.

## INTRODUCTION
Trifluralin, a pre- planting soil incorporation herbicide of the dinitroaniline group, is used widely as a weed control in soybean (*Glycine Max* L.) fields in Golestan province, in northern Iran.

Damping off caused by *Rhizoctonia solani* Kuehn (anastomosis group 4; teleomorph: *Thanatephorus cucumberis*) is a common disease in the soybean fields of this province (Hamdollah-Zadeh and Rahimian, 1989). The fungus causes damping off, root rot and aerial leaf blights on soybean around the world (Doupnik, 1993). Soybean stand and yield losses up to 50% by *R. solani* have been reported by Sharon *et al*. (1992).

Trifluralin is strongly adsorbed to organic matter and clay, with low to negligible leaching potential, and its average field half-life is 45 days on most soils (Vencil, 2002). Studies have shown that certain herbicides can lead to an increase in root disease in various crops (Sanogo *et al*., 2000; Altman & Rovira, 1989; Bauske and Kirby, 1992). An increased incidence of Rhizoctonial damping off on cotton grown in soil treated with trifluralin has been reported (Chandler and Santleman, 1967).

There is little knowledge about the effects of herbicides on interaction of disease caused by *R. solani*. The objective of this research was to study the effects of the herbicide trifluralin on the growth and virulence of *R. solani* (isolate anastomosis group 4), and the susceptibility of soybean cv. Williams to the pathogen.

## MATERIALS AND METHODS
To determine the effect of trifluralin (Treflan EC 48%) on the vegetative growth of *R. solani* AG4, the herbicide was mixed with autoclaved malt agar as it was solidifying (about 30°C). The concentration of trifluralin in the medium was adjusted to 0, 50, 100 or 200 ppm. Petri dishes of the treated medium were inoculated centrally with a 5- mm disc from a stock culture of *R. solani* AG 4 and incubated at 24°C. The experiment was

arranged as completely randomized design with six replicates and conducted twice. The growth of fungal colonies was determined by measuring their diameter in two directions at right angles after four days.

To study the effect of trifluralin on the virulence of *R. solani*, the fungus was grown on malt agar containing 0, 50, 100 or 200 ppm trifluralin, with six petri dishes of each. After seven days incubation at 24 °C in dark, fungal colonies from each treatment were harvested by gently scraping with a razor blade, The resultant product was blended in 60 mL distilled water using a mixer to produce a suspension of mycellial fragments. The suspension from each treatment was incorporated uniformly with autoclaved sand:soil (1:1, v/v) to approximately 3- cm depth in 10.5 cm diameter pots. Thus, each pot contained 10 mL of the suspension. Soybean was planted at five seeds per pot. The pots were placed in a controlled environment chamber with thermo-period cycle of 26°C for 16h and 20°C for 8h (day/night). The treatments were completely randomized with six replications and the experiment was conducted twice. According to Harikrishman and Yang (2002), plant stand was determined by counting the number of plants after 14 days, thus the differences among various treatments were assumed to be due to damping off.

A third experiment was conducted to show if trifluralin enhanced the susceptibility of soybean to the pathogen. Soybean seedlings were grown in 10.5 cm diameter pots containing an autoclaved mixture of sand:soil (1:1 v/v) which was treated with trifluralin at 0, 0.5, 1 and 2 µg ai $g^{-1}$ soil. Each pot contained five seedlings with six replications. After seven days, the seedlings were removed lightly, washed repeatedly with distilled water and replanted in pots containing a mixture of soil:sand (1:1v/v) which was inoculated with *R. solani* (as explained above) after autoclaving. The experiment was designed completely randomized with six replications and conducted twice. The pots were placed in a controlled environment chamber with thermo-period cycle of 26°C for 16h and 20°C for 8h (day/night). The seedlings in pots were checked daily up to 21 days to determine the percentage of plants lost to damping off. To confirm the disease agent, the pathogen was re-isolated from infected tissues.

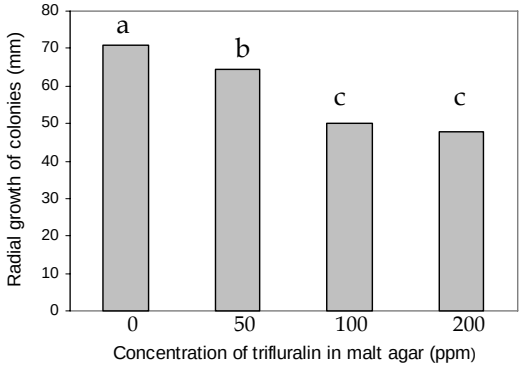


**Fig 1. Radial growth of *Rhizoctonia solani* AG 4 on malt agar containing 0 (control) to 200 ppm trifluralin, after four days incubation at 24 °C. Values are the average of six replicates. Columns with the same letter at the top are not significantly different. P≤0.05.**

**Data analysis**

For each part of investigation, data of two experiments were combined and subjected to analysis of variance in a completely randomized design using MSTAT-C procedures. Means of different treatments for various variables were compared using Duncan's multiple ranges test..

## RESULTS AND DISCUSSION

Vegetative growth of *R. solani* AG4 in malt agar containing 50, 100 or 200 ppm trifluralin was significantly reduced compared to that of untreated medium. This difference was more pronounced at higher concentrations of the herbicide (Fig 1). The response of fungi to different herbicides is varied. For example, application of glyphosate has been shown to support increase populations of several fungi in soil (Levesque *et al.*, 1987; Sinclair and Backman, 1989).

Fungal colonies grown on malt agar containing trifluralin at different concentrations caused no significant differences in the incidence of soybean damping off (Fig 2). So, There was no evidence that trifluralin had any effect on the virulence of the pathogen.

Despite the inhibitory effect of trifluralin on *R. solani* AG 4 growth, the incidence of soybean damping off in treated soil with this herbicide at 0.5, 1 and 2 µg ai $g^{-1}$ soil was 25, 37 and 42% respectively (Fig 3). Whereas damping off in untreated soil was 11.6%,

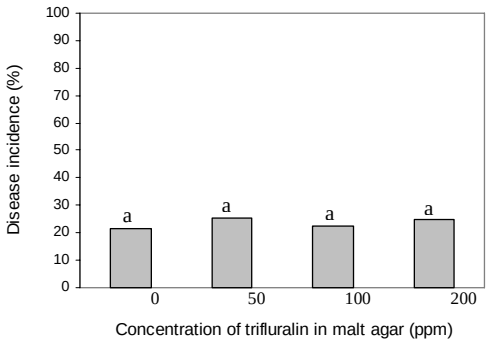


**Fig 2. Percentage of infected seedlings in soil inoculated with fungal colonies grown on malt agar containing 0 (control) to 200 ppm trifluralin, 14 days after planting. Values are the average of six replicates, each containing five seedlings. Columns with the same letter at the top are not significantly different. P≤0.05.**

significantly lower than in the trifluralin-treated soil. This shows that trifluralin enhances the susceptibility of soybean cv Williams to *R. solani* GA 4.

Herbicide-induced stresses in conjunction with physical factors have been shown to increase the predisposition of a crop to disease and fungal colonization (Altman & Rovira, 1989; Bauske & Kirby, 1992). The results of current investigation support the findings of Wiley and Ross (1974) and Harikrishman and Yang (2002) who found a significant increase in Rhizoctonial root rot of soybean with the dinitroanalin herbicides trifluralin and pendimethalin. The major effects of dinitroanaline herbicides are on plant root growth, which they stop by interfering with mitosis and preventing normal cell division and cell wall formation (Morejohn *et al*., 1987; Ross and Lembi, 1999).

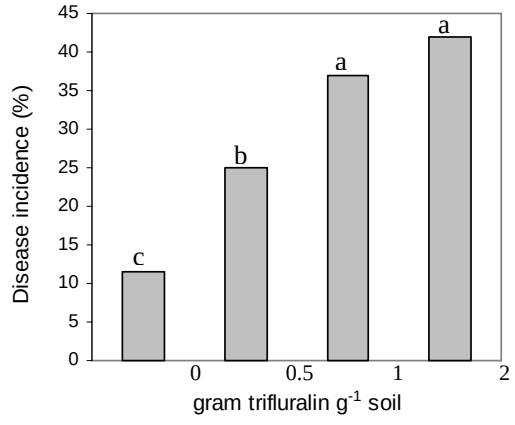


**Fig 3. Percentage of damping off in soybean grown in soil containing trifluralin at various concentrations, and replanted in soil inoculated with Rhizoctonia solani AG 4. Values are the average of six replicates, each containing five seedlings. Columns with the same letter at the top are not significantly different. P≤0.05.**

Harikrishman and Yang (2002) reported that damping off and root rot caused by *R. solani* was similar in both glyphosate-tolerant and glyphosate-sensitive cultivars following the application of most herbicide treatments. This work may be evidence of the same response in soybean cultivar to soil-applied herbicides. As such, avoiding the application of dinitroanaline herbicides as a means of reducing yield loss of soybean by *R. solani* AG 4 is recommended.

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