

# The effect of alcoholic extract of *Silybum marianum* leaves against some of *Rhizoctonia solani* strains

## Abstract

The antifungal activity of ethanol extract of *Silybum marianum* was investigated *in-vitro* against three strains of *Rhizoctonia solani* at different concentrations (5, 10, 15 and 20%). Bioassay of extract was conducted by poisoned food technique with three replications. The findings showed that strain of *R. solani* (Eggplant) was susceptible to concentrations of *S. marianum* more than other strains in mycelial growth and inhibition percentage of fungal growth (3.9cm, 56.4%) respectively whereas means of concentrations resulted to reduce the fungal growth gradually with increase concentration of *S. marianum* (5.7, 4.4, 4.3 and 1.6cm) for concentrations of (5, 10, 15 and 20%) respectively, A concentration of 20% was recorded maximum of inhibition percentage of mycelial growth of *R. solani* strains 82.2% followed by concentrations of 15, 10 and 5% that recorded 52.2, 50.3 and 36.2% respectively. The interaction between concentrations of *S. marianum* with strains of *R. solani* was showed significantly differences between them in mycelial growth and inhibition percentage of fungal growth for all *R. solani* strains.

**Keywords:** *silybum marianum*, *rhizoctonia solan*, poisoned food technique

Volume 5 Issue 3 - 2017

Hussein Ali Salim,<sup>1</sup> Mohammed Nadeem Kasim Hantoosh,<sup>2</sup> Firas Tariq Rashid,<sup>1</sup> Nadir Flayh Ali<sup>2</sup>

<sup>1</sup>Ministry of Agriculture, Iraq

<sup>2</sup>College of Agriculture, University of Diyala, Iraq

**Correspondence:** Hussein Ali Salim, Ministry of Agriculture, Baqubah-Diyala-Iraq, Iraq, Tel +964707532321, Email h\_salim1111@yahoo.com

**Received:** August 04 2017 | **Published:** August 22, 2017

## Introduction

*R. solani* is the most widely known and most studied species of genus Rhizoctonia. It was originally described by Julius Kühn from potato in 1858.<sup>1</sup> *R. solani* is soilborne Basidiomycete occurring worldwide. Its highly destructive lifestyle as a non-obligate parasite causes necrosis and damping-off on numerous host plant species. Because of the lack of conidia, *R. solani* exists as vegetative hyphae and sclerotia in nature. Sclerotia are an encapsulated, tight hyphal clump that protects and preserves the fungus over non-optimal times. The fungus is dispersed mainly via sclerotia, contaminated plant material or soil spread by wind, water or during agricultural practices such as tillage and seed transportation.<sup>2</sup> Phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on the environment.<sup>3</sup> Continuous application of such chemicals has negative impacts on human health and destruction of the ecosystem that results in new strains of pathogens that are more resistant and difficult to control.<sup>4</sup> It has become necessary to adopt eco-friendly approaches for better crop health and for yield. In the past, several higher plants have proved their usefulness against a number of fungi.<sup>5</sup> Plant extracts have the ability to inhibit mycelial growth in many fungal species.<sup>6-8</sup> *Silybum marianum* (L), milk thistle, is an annual or biennial herb. It belongs to the family Asteraceae.<sup>9</sup> The plant is known for its medicinal properties having important chemical constituents including several flavonolignans collectively known as silymarin that has antioxidant properties.<sup>10</sup> In the present study, the antifungal activity of leaves extract of *S. marianum* was tested *in vitro* against the *R. solani* strains that isolated from the plants (tomato, eggplant, and cauliflower).

## Materials and methods

A laboratory experiment was carried out in a college of agriculture, university of Diyala during 2016. The *S. marianum* leaves were collected from orchard located in Baqubah city, *R. solani* strains were isolated from infected plants (tomato, eggplant and cauliflower). Leaves of *S. marianum* were thoroughly washed under tap water

to remove dust and other impurities then dried and powdered with the help of a blender to a fine powder, 250gm powder was soaked with 500ml of ethanol for preparing the alcoholic extract and kept on shaker about 24hour for continuous stirring, the extract was filtered by double layered muslin cloth and the extracts were centrifuged at 3000 rpm for 10 minutes. Supernatant was dried by oven at 40°C to evaporate the solvent and it was turned to solid. Concentrations of extract 5, 10, 15, 20% were prepared and poured into Petri dishes by poison food technique,<sup>11</sup> then allowed to solidify. The Petri dishes were inoculated with the pathogen by transferring five mm diameter agar disc from the fresh cultures. Three replications were maintained for each treatment. The basal medium (PDA) without any plant extract served as the control, all the inoculated Petri dishes were incubated at 25±1°C for seven days. The cross diameters of colony growth of test fungus was measured in all the treatments and compared with the control. The percent inhibition of fungal growth was estimated by using following formula.<sup>12</sup>

$$I = \frac{C - T}{C} \times 100$$

Where, I=percent inhibition, C=Colony diameter in control and T=Colony diameter in treatment.

The experiment was conducted in Factorial Experiments and the data was analyzed by one way Analysis of Variance (ANOVA).<sup>13</sup>

## Results and discussion

The data revealed that means of *R. solani* strains were significantly difference in mycelial growth of *R. solani* at all tested concentrations of *S. marianum*, *R. solani* (Eggplant) was susceptible to concentrations of *S. marianum* more than other strains (3.9cm) whereas means of concentrations resulted to reduce the fungal growth gradually with increase concentration of *S. marianum* (5.7, 4.4, 4.3 and 1.6cm) for concentrations of (5,10,15 and 20%) respectively, interaction between concentrations of *S. marianum* with strains of *R. solani* was

significantly difference in reduction of fungal growth of *R. solani* (Table 1 & Figures 1-3). *R. solani* (Eggplant) was susceptible to concentrations of *S. marianum* in inhibition percentage of fungal growth at (56.4%) followed by *R. solani* (Tomato) 43.5% and *R. solani* (cauliflower) 32.6%, concentrations of *S. marianum* showed significant activities in percent inhibition of fungal growth of *R.*

*solani* . A concentration of 20% was recorded maximum of inhibition percentage of mycelial growth of *R. solani* strains 82.2% followed by concentrations of 15, 10 and 5% that recorded 52.2, 50.3 and 36.2% respectively. The interaction between concentrations of *S. marianum* with strains of *R. solani* was showed significantly differences between them (Table 2).

**Table 1** *In vitro* efficacy of ethanol extract of different concentrations of *S. marianum* on fungal growth of *R. solani* strains

Strains of <i>R. solani</i>	Concentrations of <i>Silybum marianum</i>					Means of strains (cm)
	5%	10%	15%	20%	Control	
<i>R. solani</i> (Tomato)	5.2	5	4.9	1.3	9	5
<i>R. solani</i> (Eggplant)	5.4	2	1.8	1.4	9	3.9
<i>R. solani</i> (cauliflower)	6.6	6.4	1.8	2.1	9	6
Means of Concentrations (cm)	5.7	4.4	4.3	1.6	9	



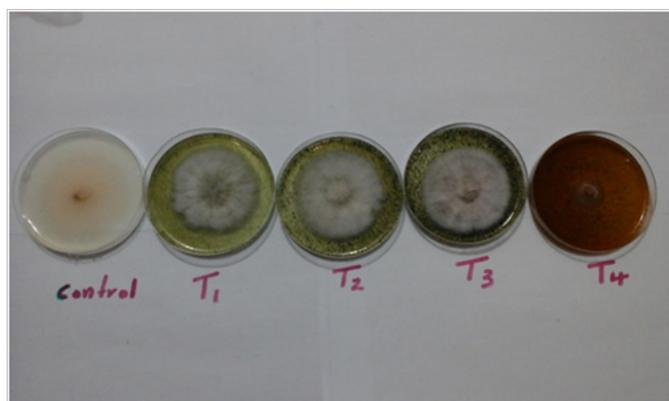
**Figure 1** Efficacy of ethanol extract of different concentrations of *S. marianum* on *R. solani* (Tomato).



**Figure 2** Efficacy of ethanol extract of different concentrations of *S. marianum* on *R. solani* (Eggplant).

**Table 2** *In vitro* efficacy of ethanol extract of different concentrations of *S. marianum* in percent inhibition of fungal growth of *R. solani* strains

Strains of <i>R. solani</i>	Concentrations of <i>Silybum marianum</i>					Means of strains (cm)
	5%	10%	15%	20%	Control	
<i>R. solani</i> (Tomato)	42.2	44.4	45.5	85.5	0	5
<i>R. solani</i> (Eggplant)	40	77.7	80	84.4	0	3.9
<i>R. solani</i> (cauliflower)	26.6	28.8	31.1	76.6	0	32.6
Means of Concentrations %	36.2	50.3	52.2	82.2	0	



**Figure 3** Efficacy of ethanol extract of different concentrations of *S. marianum* on *R. solani* (Cauliflower).

CD (0.05) Concentrations 0.3

CD (0.05) Strains of *R. solani* 0.2

CD (0.05) Concentrations×Strains of *R. solani* 0.6

*S. marianum* extract has antifungal effects and preventing the growth of fungi.<sup>10</sup> *S. marianum* extract contains 70% silymarin, a mixture of the silibinin and flavonolignans. So these phytochemicals may also be toxic to bacterial cell and may be responsible for the antibacterial activity of *S. marianum*.<sup>14</sup> *S. marianum* sprouts are rich in polyphenols and demonstrate antioxidative capacity.<sup>15</sup> Plant activity of *S. marianum* might be due to the presence of tannins and flavonoids that acting as primary antioxidants.<sup>16</sup> Oxidative stress induced by pathogen infection is sensed by plants in the form of activation of reactive oxygen species which subsequently activates plant defenses.<sup>17,18</sup>

Rate of inhibition

CD (0.05) Concentrations 0.9

CD (0.05) Strains of *R. solani* 0.7

CD (0.05) Concentrations×Strains of *R. solani* 1.6

## Conclusion

Under *in vitro* conditions, the strains of *R. solani* were well controlled by ethanol extract of *S. marianum* by different concentrations. This extract showed maximum activity; even at very low concentrations. The extract of *S. marianum* could be considered as potential source of antifungal compound for treating *R. solani* strains. We conclude from that this extract exhibit fungicidal properties that support their use as antiseptics. These need to be further evaluated under *in vivo* conditions so as to make minimum use of chemicals.

## Acknowledgements

None.

## Conflict of interest

The author declares no conflict of interest.

## References

- Kühn J. *Die Krankheiten der Kulturwachse, ihre Ursachen und ihre Verhütung*. Germany: Gustav Bosselman; 1858. 312 p.
- Deacon JW. Translocation and transfer in *Rhizoctonia*: mechanism and significance. In: Sneh B, Jabaji-Hare S, editors. *Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control*. Netherlands: Kluwer Academic Publishers; 1996. p. 117–125.
- Harris CA1, Renfrew MJ, Woolridge MW. Assessing the risk of pesticide residues to consumers: recent and future developments. *Food Addit Contam*. 2001;18(12):1124–1129.
- Khalil AB, Dabaneh BF, Anfoka GH. Antifungal Activity of Medicinal Plants from Jordan Environment. *Plant Pathol J*. 2005;4(2):130–132.
- Anil S, Saroj A, Parminder K. Evaluation of plant extracts against *Rhizoctonia solani* causing sheath blight of rice. *The Journal of Plant Protection Sciences*. 2009;1(1):25–30.
- Guerin JC, Reveille HP. Antifungal activity of plant extracts used in therapy. I. Study of 41 plant extracts against 9 fungi species. *Annales Pharmaceutiques Francaises*. 1984;42:553–559.
- Natarajan MR, Lalithakumari D. Antifungal activity of the leaf extracts of *Lawsonia inermis* on *Drechslera oryzae*. *Indian Phytopathology*. 1987;40:390–395.
- Singh RK, Dwivedi RS. Effect of oils on *Sclerotium rolfsii* causing foot-rot of barley. *Indian Phytopathology*. 1987;40:531–533.
- Nasir YJ. *Flora of Pakistan: Asteraceae*. Islamabad: National Herbarium Pakistan Agriculture Research Council; 1990. 192:59–117.
- Salehi M, Hasanloo T, Mehrabian S, et al. Effects of *Silybum marianum* (L.) Gaertn seeds extract on dermatophytes and saprophytes fungi *in vitro* compare to clotrimazol. *Pharmaceutical Sciences*. 2011;16(4):203–210.
- Nene VL, Thaplyal PN. *Fungicides in Plant Disease Control*. India: Oxford & IBH Publ. Co. Pvt. Limited; 1987. 507 p.
- Vincent JM. Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*. 1947;15:850.
- Fisher RA, Yates. *A statistical method for research workers*. UK: Oliver and boyd ltd; 1968. 10 p.
- Amoos Bajwa, Sheraz Tariq, Alamgeer Yuchi, et al. Evaluation of anti-bacterial activity of *Silybum marianum* against pathogenic and resistant bacteria. *European journal of medicinal plants*. 2016;13(4):1–7.
- McDonald S, Prenzler PD, Antolovich M, et al. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. 2001;73:73–84.
- Kousara S, Hicksb LM, Sheikha MA, et al. Bioactive potential of stress induced extracts of *silybum marianum*. *Biological and biochemical oxidation processes*. 2015;38(4):1678–1689.
- Bolwell GP, Bindschedler LV, Blee KA, et al. The Apoplastic Oxidative Burst in Response to Biotic Stress in Plants: A Three Component System. *J Exp Bot*. 2002;53(372):1367–1376.
- Sayyah M, Boostania H, Paksereshta S, et al. Comparison of *Silybum marianum*(L.) Gaertn. With fluoxetine in the treatment of Obsessive–Compulsive Disorder. *Prog Neuropsychopharmacol Biol Psychiatr*. 2011;35(4):887–895.