

Original Research Article

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Efficiency of Two Common Spices against Green Gram Pathogen *Rhizoctonia bataticola*

Aarti R. Deshpande*

Department of Microbiology, Shankarlal Khandelwal College, Akola (Maharashtra), India

*Corresponding author

ABSTRACT

Exploring eco-friendly fungitoxicants is a current need in the context of hazardous effects of chemical fungicides. In the present work, inhibitory effect of two common spices viz. cinnamon and garlic was evaluated against *Rhizoctonia bataticola*, a common soil borne pathogen of green gram. The inhibition of *R. bataticola* growth with different concentrations of both these spices was in the range of 26.25% to 100% by poisoned food technique. The inhibitory effect of different concentrations of carbendazim and copper oxychloride was in the range of 43.75% to 100%. Autoclaving of the medium along with spices resulted in increase in growth inhibition of the pathogen as compared with the inhibition due to spices when added after autoclaving. Aqueous extracts prepared with boiling were more effective than the extracts prepared without boiling. Antagonistic potential of the spices was evaluated in terms of increase in seedling vigor index (SVI) of green gram seeds inoculated with *R. bataticola* by paper towel method. The increase in SVI was in the range of 25.85% to 45.03% with independent treatments of garlic and cinnamon to the pathogen inoculated seeds. SVI of the pathogen inoculated seeds increased by 30.43% with 1% carbendazim.

Keywords

Antagonistic activity, *Cimomomum zeylanicum*, *Rhizoctonia bataticola*, Green gram, Cinnamon, Garlic, Antifungal activity

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Introduction

A wide spread use of chemical fungicides for controlling phytopathogens has created imbalance in agricultural ecosystems leading to deterioration of soil health and productivity (Walia *et al.*, 2014, Bacmaga *et al.*, 2016). These chemical fungicides cause toxicity to

non-target plants, animals and microbial life (Brauer *et al.*, 2019) and also are causing extremely hazardous effects on human and animal health because of their carcinogenicity, mutagenicity and teratogenicity (Jasuja, 2015). In order to mitigate the problems due to extensive use of chemical fungicides there is growing interest

in exploring the environment friendly alternatives for sustainable agricultural practices such as biofungicides and herbal fungicides. A wide variety of plant extracts and essential oils have been evaluated for their bioefficacy against many fungal photopathogen (Isman, 2000, Bhagwat and Datar, 2014, Suprapta 2016, Zambonelli, 2008).

Green gram (*Vignaradia L.*) is a legume crop with large cultivation in Asia and is a major crop in many states of India including Maharashtra. Fungal diseases cause yield losses of green gram upto 46-60% (Kaur *et al.*, 2011). The soil and seed borne fungus *Macrophomina phaseolina* (Pycnidial stage of *Rhizoctonia bataticola*) causes root rot, collar rot, stem rot and leaf blight in green gram. Agallol, Captan, Thiram, Carbendazim and Thiophanate – methyl and benonyl are the chemical fungicides that have been evaluated to control *Macrophomina phaseolina* (Swamy *et al.*, 2018).

In some studies plant extracts and their products have been assessed against green gram pathogens. (Javed and Amin, 2009, Murugapriya *et al.*, 2011). Control of *Macrophomina* growth in- vitro and its incidences in green house conditions using plant based materials and their combination with other chemicals have also been reported (Pandey *et al.*, 2018).

Garlic (*Allium sativum*) is a commonly used spice since ancient times and a medicinal herb that has wide spectrum of antimicrobial action (Harris *et al.*, 2001). Similarly Cinnamon (*Cinnamomum zeylanicum*) is also a widely used spice having antibacterial (Nawabi *et al.*, 2015) and antifungal properties (Ranshinghe *et al.*, 2013)

The cost of production of any crop is directly influenced by the cost and amount of

agricultural inputs. Overuse of chemical agro-inputs increase the cost of crop production. The present work has been undertaken to evaluate the antifungal potential of two common species cinnamon and garlic against *Rhizoctonia bataticola*, a common soil borne pathogen of green gram.

An attempt has been made with the objective of assessing the fungi-toxic effect of powder of cinnamon, juice of garlic and the aqueous extracts of these spices which may lead to development of simple domestic method of seed treatment to prevent crop loss due to seed and soil borne fungal phytopathogens.

Materials and Methods

Poisoned Food Technique

Requisite quantity of herbal powder/ extract / chemical fungicide was added in sterilized melted (45⁰C) PDA separately so as to get test concentration. Chloramphenicol was added to prevent bacterial contamination. Flasks containing poisoned medium were shaken well to have even and uniform distribution of herbals and chemical fungicides and poured in petriplates. Fungal disk of 5 mm diameter cut from one week old fungus lawn culture was placed in the centre of the petriplate containing poisoned medium.

The control plate was kept where the fungal disc was grown under same conditions on plain PDA. Diameter of fungal growth was recorded on 3rd, 4th, and 5th day. The Vincent formula was used to calculate the percentage of inhibition of fungal growth.

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

Where

I = % inhibition of growth

C = Growth of fungus in control

T = Growth of fungus in treatments

Preparation of aqueous extracts

Total 25 g of each herbal powder was added in 200 ml of distilled water and the extraction was carried out with boiling and at room temperature with shaking. The extracts were filtered through blotting paper and used for antifungal activity testing at three concentrations.

Evaluation of Seedling Vigour Index

Germination paper method

The seeds were germinated between two layers of germination papers which are placed in glass or plastic containers filled with water in required quantity.

The paper was not so wet that if pressed a film of water forms around the finger. Seeds were infected with fungal spores for maximum 2 hours and then treated with different concentrations of herbals and chemical antifungal agents for 30min. Seeds were arranged on the germination paper and covered

with another sheet of germination paper. Then the papers were rolled carefully and the rolls were dipped in container containing water for 10 days. After 10 days the results were noted in terms of % germination, average root length & average shoot length. Seeds without inoculation of fungal pathogen and without treatment of antifungal agents served as control. Seedling vigor index were calculated for each treatment and control.

$$\text{Seedling Vigour Index} = \% \text{ germination} \times (\text{Root length} + \text{Shoot length})$$

Results and Discussion

Antifungal activity of *C. zeylanicum* bark powder was better than that of *A. sativum* juice of cloves at 0.1% and 0.5% concentration by poisoned food technique (Table-1). *C. zeylanicum* activity was better than *A. sativum* activity by 29.21% at 0.1% concentration and by 63.53% at 0.5% concentration of the fungitoxicant added before autoclaving.

Table.1 Effect of autoclaving on the antifungal activity of herbal and chemical fungi toxicants against *Rhizoctonia bataticola* by poisoned food technique.

S.No.	Fungitoxicant	Concentration	After autoclaving	Before autoclaving
1	<i>A.sativum</i>	0.1%	nm	29.41
2	<i>A.sativum</i>	0.5%	nm	36.47
3	<i>C. zeylanicum</i>	0.1%	26.25	41.18
4	<i>C. zeylanicum</i>	0.5%	48.75	100
5	Carbendazim	0.1%	43.75	52.94
6	Carbendazim	0.5%	100	100
7	Copper oxychloride	0.1%	78.75	81.17
8	Copper oxychloride	0.5%	100	100

Note : nm=Not measurable

Table.2 Efficacy of herbal and chemical fungi toxicants on SVI of green gram seeds inoculated with *Rhizoctonia bataticola*.

S.No.	Fungitoxicant	Concentration	Germination (%)	ARL (cm)	ASL (cm)	SVI
1	Control	-	78	8.65	5.51	1164.48
2	Fungus (F) infected	-	49	8.29	0.06	763.15
3	(F) + <i>A. sativum</i>	1%	70	9.47	5.53	1050
4	(F) + <i>A. sativum</i>	10%	66	9.66	4.75	948.42
5	(F) + <i>C. zeylanicum</i>	1%	72	10.45	7.31	1279.38
6	(F) + <i>C. zeylanicum</i>	10%	68	9.65	6.82	1120.79
7	(F) + (<i>A. sativum</i> + <i>C. zeylanicum</i>)	10%	76	9.60	7.67	1312.52
8	(F) + Carbendazim	1%	68	9.35	6.77	1096.99

Note: ARL- Average root length, ASM- Average shoot length, SVI-Seedling Vigor Index

The antifungal activities of carbendazim and copperoxychloride were better than the herbal fungitoxicants at 0.1% concentration whereas at 0.5% concentration 100% inhibition was obtained by *C.zeylanicum* and both the chemical fungitoxicants. Among the two chemical fungitoxicants, copper oxychloride was better than carbendazim at 0.1% concentration (Table-1).

The inhibitory activity of spices were far better when the powders were added before autoclaving than the inhibitory activity of spices added after autoclaving. This indicates better extraction of fungitoxicants at high temperature with pressure. Moreover the fungitoxic components were thermostable.

Kishore and Pande in 2007 has reported the broad spectrum antifungal activity of cinnamon essential oil against 14 different phytopathogenic fungi including *R. bataticola*. Ransinghe *et al.* (2002) have also reported the antifungal activity of *C. zeylanicum* against some other plant pathogenic fungi. Most of such studies were

focused on the fungitoxic potential of essential oil of *C. zeylanicum*. Present studies were carried out using powder of commercially available bark of *C. zeylanicum* from farmer's point of view.

Kutawa *et al.*, (2018) had assessed the fungitoxic potential of *A. sativum* against *Fusarium* and *Rhizopus* and had reported the superior action of its ethanolic extract than aqueous extract. Present investigations demonstrated the inhibitory potential of *A. sativum* juice of cloves at 0.1% concentration against *R. bataticola* when added before autoclaving of the medium.

Aqueous extracts of the spices were inhibitory for the growth of *R. bataticola*. The extract prepared with boiling outperformed the extracts prepared without boiling (Figure -1, Plate-1). The inhibitory potential of *C. zeylanicum* extracts was better than that of *A. sativum* extracts. *C. zeylanicum* 10% extract prepared with boiling resulted in highest degree of inhibition (95.18%) among all the tested fungi toxic preparations.

Effect of independent and combined seed applications of *C. zeylanicum* powder and *A. sativum* juice was assessed on the seedling vigor index (SVI) of *R. bataticola* inoculated green gram seeds by paper towel method (Table -2). Pathogen inoculation resulted in 36.33% decrease in SVI over control.

Treatment of the pathogen inoculated seeds with *A. sativum* and *C. zeylanicum* at 1% and 10% concentration by weight of seeds increased SVI in the range of 25.85% to 45.03% over infected control. It was interesting to note that the performance of 1% spices concentration was better than that of 10% spices concentration suggesting that high concentration of spices may have been detrimental for the process of germination and seedling growth.

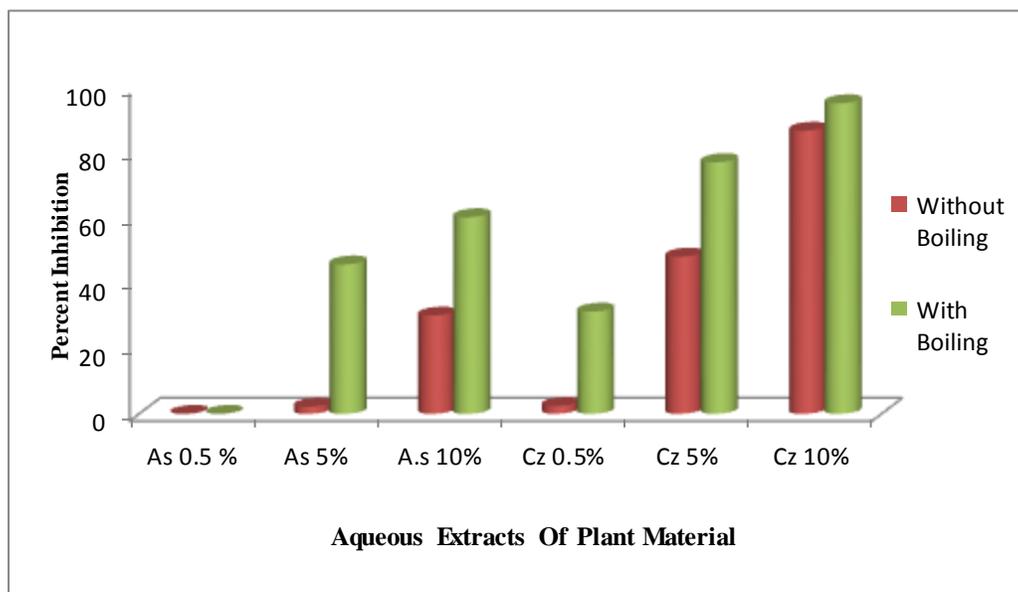
Combined treatment with spices at 10% concentration of 1:1 mixture resulted in 45.35% increase in SVI whereas independent treatment of *A. sativum* (10%) and *C. zeylanicum* (10%) produced 25.85% and 37.26% increase in the SVI of pathogen inoculated green gram seeds

respectively (Figure-2).

All the above findings suggest that independent seed treatment with both the spices at 1% concentration appeared to be effective in overcoming *R. bataticola* infection of green gram seeds. Their combined seed application also appeared to be effective.

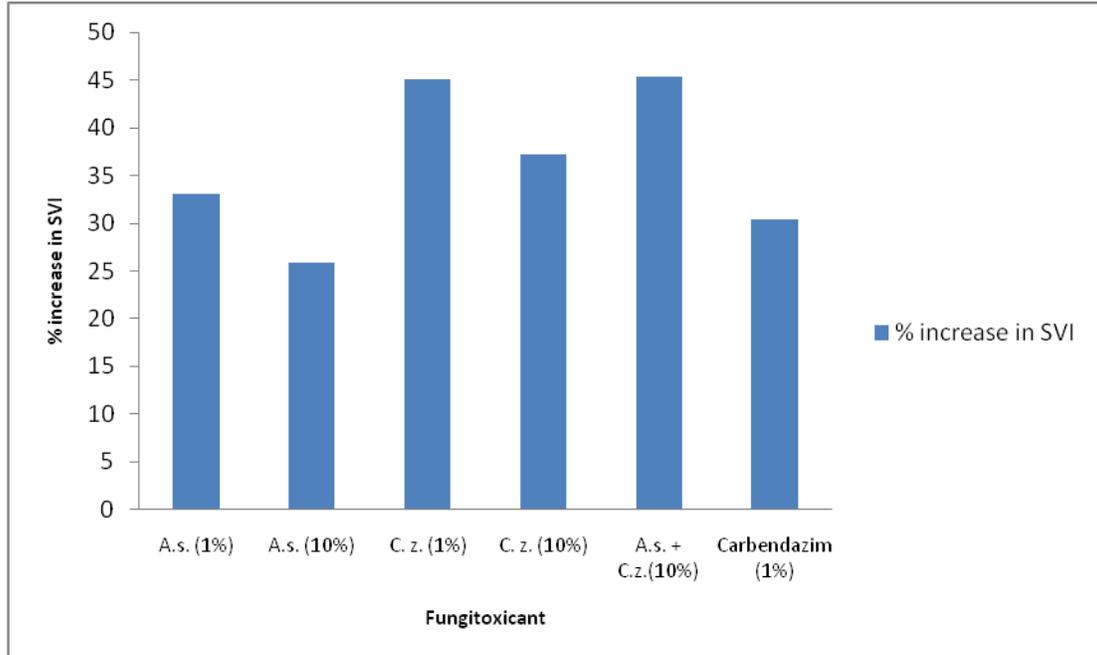
Kishore and Pande (2007) had tested foliar spray, seed treatments and soil amendment of *C. zeylynicum* essential oil for control of late leaf spot and crown rot diseases of peanut and had found only soil amendment as effective method for the disease control. They had stated the impracticality of the soil amendment with essential oil.

Present work demonstrated the efficacy of independent or combined seed treatment with garlic and/or cinnamon for increase in SVI of *R. bataticola* inoculated green gram seeds. However intensive studies are required in future to validate the application potential of seed treatment with these spices under field conditions.



Note: *As*=*Allium sativum* *Cz.* = *Cinnamomumzeylanicum*

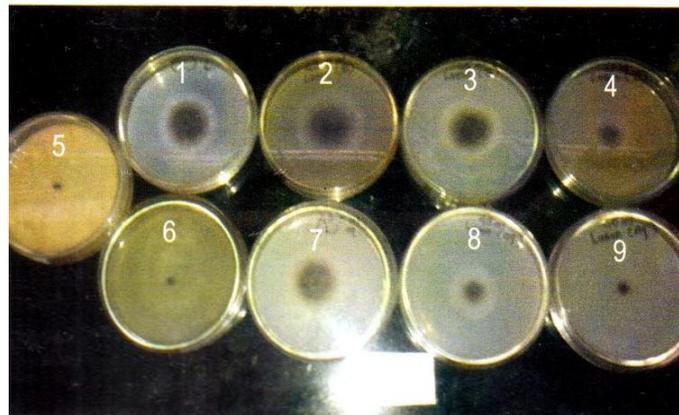
Figure.1 Antifungal activity of aqueous extracts against *Rhizoctonia bataticola*



Note : A.s. = *Allium sativum* C.z. = *Cinnamomumzeylanicum*

Figure.2 Percent increase in SVI of *R. bataticola* inoculated seeds with different treatments.

Plate.1 Antifungal activity of aqueous extracts of *Allium sativum* and chemical fungitoxicants against *Rhizoctonia bataticola* by poisoned food technique



Control ; 2,3,4-0.5% ,5% and 10% extract prepared without boiling respectively
5-0.5% Carbendazim; 6-0.5% Copperoxychloride; 7,8 ,9 -0.5% ,5% and 10% extract prepared with boiling respectively

Cinnamon powder and garlic juice as well as their aqueous extracts were effective for growth inhibition of *R. bataticola* in-vitro. Autoclaving or boiling considerably improved the performance of the spices regarding their inhibitory activity against the pathogen. Seed

treatment with the powder of cinnamon and the juice of garlic were effective for increasing SVI of the pathogen inoculated green gram seeds under laboratory conditions. Combined application of garlic and cinnamon 1:1 mixture at 10% concentration was also an

effective treatment which produced improvement in SVI of the pathogen inoculated green gram seeds under experimental conditions.

Their performance under field conditions needs to be assessed in order to validate their application potential.

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