JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 941-946

Study on Antifungal Activity of *Zingiber officinale* Rosc. and *Allium sativum* L. Against some Pathogenic Fungi

Anand Sagar*, Shrinkhala Guleria and Bhawana Srivastava

Department of Bio-Sciences, Himachal Pradesh University, Shimla - 171 005, India.

(Received: 29 November 2010; accepted: 10 January 2011)

Aqueous extract of ginger (*Zingiber officinale* Rosc.) rhizome and garlic (*Allium sativum* L.) bulb was evaluated for its anti-mycotic activity against *Alternaria alternate* [Fr.] Keissl, *Aspergillus flavus* Link and *Fusarium solani* F. Sp.pisi adopting Poisoned food technique using Potato dextrose agar (PDA) medium at 26±2°C. The ginger extract alone as well as in combination with garlic extract showed significant reduction in the growth of the tested fungi at 5, 10, 15 and 20% concentration (conc.).The two plant extracts were also evaluated for its fungitoxicity (fungistatic and fungicidal) and exhibited broad fungitoxic spectrum. Further work involves further evaluation of antifungal activities of these extracts *in vivo*.

Key words: Rhizome extract, Bulb extract, Antifungal activity, Test fungi.

Agricultural production of the world sustains annual loss of about 20 to 30% on an average due to plant diseases in different crops and in different countries. The use of synthetic chemicals as anti-microbial for the management of plant diseases has undoubtedly increased crop protection but with considerable deterioration of environmental quality and human health[1]. Thus, the danger inherent in the use of agrochemicals has brought forth the need to explore other safer alternatives. An attempt was made to employ plant extracts against number of plant pathogens. A large number of plants have been reported to possess fungitoxic properties against plant pathogens which could be exploited commercially with practically no residual or toxic effect on ecosystem[2,3]. The plant extract will help in reducing cost, environmental hazards and development of resistance by pathogen to fungicides. In present investigation, rhizome extract of ginger (*Zingiber officinale* Rosc.) and garlic (*Allium sativum* L.) bulb were evaluated at four conc. (5, 10, 15 and 20%) against *Alternaria alternate* [Fr.] Keissl, *Aspergillus flavus* Link and *Fusarium solani* F. Sp.pisi under *in vitro* condition.

MATERIAL AND METHODS

Procurement of plant material

Fresh rhizomes of *Z. officinale* and bulbs of *A. sativum* were collected from Sanjauli area of Distt. Shimla (H.P.). These were identified by Prof. M.K. Seth (Wood sciences, forest biodiversity and plant resources laboratory), Department of

* To whom all correspondence should be addressed.
Tel.: 91-9816807646, 91-177-2831947
E-mail: as_bio@rediffmail.com

Biosciences, Himachal PradeshUniversity, Shimla.

Procurement of fungal cultures

The toxigenic strain (Saktiman 3Nst) of *A.flavus* was procured from Herbal pesticide laboratory, Centre of Advance study in Botany, Faculty of Science, Banaras Hindu University, Varanasi. *A. alternata* and *F. solani* pure samples was procured from Plant Pathology Department, Central Potato Research Institue, Shimla.

***In vitro* evaluation of plant extracts**

Plant materials were washed first under tap water and then in distilled water. 100 g of each fresh sample was chopped and then crushed separately in a surface sterilized pestle and mortar by adding 100 ml sterile distilled water (1:1 w/v). The two extracts were filtered through two layers of muslin cloth followed by Whatman filter paper no. 1. Finally two filtrate thus obtained was used as stock solutions. To study the antifungal mechanism of plant extract Poisoned food technique was used[4]. 5, 10, 15 and 20 ml of stock solution was mixed with 95, 90, 85 and 80 ml of sterilized molten Potato dextrose agar (PDA) media, respectively so as to get 5, 10, 15 and 20% conc. of ginger extract alone as well as its combination with garlic extract. The medium was thoroughly shaken for uniform mixing of extract. Ten ml of medium was poured into sterile petriplates (9 cm diameter) and allowed to solidify. Discs of each test fungi were cut from periphery of actively growing seven day old culture by sterile cork borer and one such disc of each was placed on the centre of each agar plates in sets of three. Controls were also maintained by growing pathogen on PDA plates containing requisite amount of distilled water in place of extracts. The petriplates were incubated at 26±°C for seven days in an incubation chamber. Diameter of fungal colonies of treatment and control sets were measured in mutually perpendicular directions on seventh day. The percentage inhibition of radial growth of the test fungus by aqueous extract was calculated following Pandey *et al* .,[5] method as:

$$\text{Percentage mycelial inhibition} = \frac{dc - dt}{dc} \times 100 ,$$

dc = average diameter of fungal colony in control sets, and *dt* = average diameter of fungal colony in treatment sets.

Nature of toxicity (fungicidal or fungistatic) of aqueous extract of *Z. officinale* and combination of aqueous extract of *Z. officinale* and *A. sativum* was determined against test fungi following Garber and Hauston[6], Thompsom[7], Srivastava[8] . Requisite amount of extract was dissolved separately in 10 ml of PDA medium to get final conc. of 5, 10, 15 and 20%. The plates were inoculated aseptically with fungal discs taken from the periphery of a seven day old culture of the test fungi and were inoculated for six days at 26±°C. On the seventh day, the inhibited discs were taken out of the plates, washed with sterilized water and reinoculated aseptically to plates containing 10 ml of fresh PDA medium. The revival of growth of fungal disc was observed and the percentage mycelial inhibition with respect to control set was calculated on seventh day.

RESULTS AND DISCUSSION

The two botanicals inhibited the mycelia growth of the three test fungi over control. Many researchers have reported on the fungal inhibitory property of *Z. officinale* and *A. sativum* [9,10] **.**The Poisoned food technique has been preferred by most of the workers for listing of antifungal activity [11,12,13,14] . The effectiveness of the extracts increased with increase in concentration of extracts and maximum inhibition of growth was recorded at 20% conc. The results are presented in Table-1& Plate-1 .

Results depicted in Table-1 indicated that, *A. alternata* (78.8%) was significantly inhibited by *Z. officinale* rhizome extract. *A. flavus* (66.6%) was next best and this was followed by *F. solani* (65.0%) at 20% conc. Although higher plants have been reported as antifungal, there is little information on antifungal activity of plant extracts when combined together [15,16,17].

It was observed that the effectiveness of ginger extract increased when combined with garlic extract, and the inhibition increased with increase in conc. of extracts and maximum inhibition of growth was recorded at 20% conc. The results are presented in Table-2&Plate-2.

Results depicted in Table-2 indicated that *A. alternata* (100%) was inhibited completely by the two extracts combination.*F. solani* (93.3%) was next best and this was followed by *A. flavus* (88.6%) at 20% conc.

Reinoculation of the test fungi showed that whether the growth of the test fungi was inhibited temporarily (fungistatic) or permanently (fungicidal). This study emphasized the practical application of the plant extract as a fungitoxicant. It is evident from Table-3&4 that only combination

Table 1. Result of fungitoxic investigation of *Z. officinale* rhizome extract against test fungi viz: *A. alternata, A. flavus* and *F. solani*

Concentration	%inhibition of growth by aqueous extract of *Z. officinale*		
(%)	*A. alternata*	*A. flavus*	*F. solani*
5	65.2±0.14	51.1±0.00	54.1±0.21
10	70.5±0.24	56.6±0.00	55.8±0.23
15	75.8±0.04	58.8±0.00	58.8±0.00
20	78.8±0.00	66.6±0.00	65.0±0.17

Each data point represents mean of three replicates ± S. E

Table 2. Fungitoxic screening of aqueous leaf extract of *Acorus calamus* + Bulb extract of *Allium sativum* against pathogenic strains of fungi *A. alternata, A. flavus* and *F. solani*

Concentration	% inhibition of growth by aqueous extract of *Z. officinale* and *A. sativum*		
(%)	*A. alternata*	*A. flavus*	*F. solani*
5	82.2±0.00	69.7 ±0.02	72.5±0.04
10	84.4±0.00	72.2±0.00	83.3±0.00
15	85.5±0.00	84.4±0.00	86.6±0.00
20	100±0.00	86.6±0.00	93.3±0.00

Each data point represents mean of three replicates ± S. E

Table 3. Nature of toxicity of aqueous rhizome extract of *Z. officinale* against *A. alternata, A. flavus* and *F. solani*

Fungi	Concentration	Percent inhibition		Nature of
	(%)	Treated	Re inoculated	toxicity
A. alternata	5	65.2±0.14	62.2±0.02	Static
	10	70.5±0.24	66.4±0.01	Static
	15	75.8±0.04	72.1±0.00	Static
	20	78.8±0.00	74.4±0.01	Static
A. flavus	5	51.1±0.00	48.8±0.01	Static
	10	56.6±0.00	51.5±0.02	Static
	15	58.8±0.00	55.2±0.00	Static
	20	66.6±0.00	62.6±0.00	Static
F. solani	5	54.1±0.21	51.4±0.02	Static
	10	55.8±0.23	52.6±0.00	Static
	15	58.8±0.00	55.2±0.12	Static
	20	65.0±0.17	62.1±0.00	Static

Each data point represents the mean of three replicates ± S.E

of rhizome extract of *Z. officinale* and bulb extract of *A. sativum* was found to be fungicidal at 20% conc. against *A. alternata*. Rest all combination treatments as well as ginger extract alone treatments were found to be fungistatic.Table 5&6 shows that rhizome extract of *Z. Officinale* and its combination

Table 4. Nature of toxicity of *Z. officinale* extract and its combination with A. sativum extract against *A. alternata, A. flavus* and *F. solani*

Fungi	Concentration (%)	Percent inhibition		Nature of toxicity
		Treated	Re inoculated	
A. alternata	5	82.2±0.00	78.8±0.01	Static
	10	84.4±0.00	80.1±0.01	Static
	15	85.5±0.00	81.8±0.00	Static
	20	100±0.00	100±0.00	Cidal
A. flavus	5	69.7±0.12	64.4±0.01	Static
	10	72.2±0.00	68.2±0.12	Static
	15	84.4±0.00	79.9±0.00	Static
	20	86.6±0.00	82.5±0.00	Static
F. solani	5	69.7±0.12	64.4±0.01	Static
	10	72.2±0.00	68.2±0.12	Static
	15	84.4±0.00	79.9±0.00	Static
	20	86.6±0.00	82.5±0.00	Static

Each data point represents the mean of three replicates ± S.E

Table 5. Fungitoxic spectrum of *Z. officinale* extract at 15% and 20% conc

Tested Fungi	% Inhibition of growth of test fungi	
	15%	20%
Alternaria alternata	75.8±0.04	78.8±0.00
Aspergillus flavus	58.8±0.00	66.6±0.00
Fusarium solani	58.8±0.00	65.0±0.17
Helminthosporium oryzae Breda de Hann	100±0.00	100±0.00
Penicillium italicum Wehmer	100±0.00	100±0.00
Pythium debarynum Hesse	100±0.00	100±0.00

Each data point represents the mean of three replicates ±SE

Table 6. Fungitoxic spectrum of combination of *Z. officinale* and *A. sativum* extracts at 15% and 20% conc

Tested Fungi	% Inhibition of growth of test fungi	
	15%	20%
Alternaria alternata	85.5±0.00	100±0.00
Aspergillus flavus	84.4±0.00	86.6±0.00
Fusarium solani	86.6±0.00	93.3±0.00
Helminthosporium oryzae Breda de Hann	100±0.00	100±0.00
Penicillium italicum Wehmer	100±0.00	100±0.00
Pythium debarynum Hesse	100±0.00	100±0.00

Each data point represents the mean of three replicates ±SE

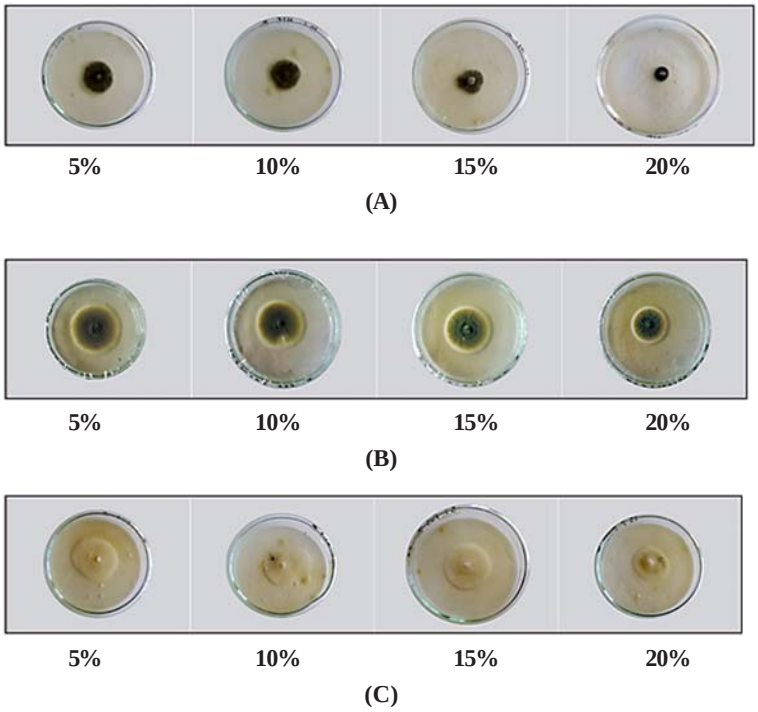

Plate 1. Percentage inhibition of growth of *Alternaria alternata* (A), *Aspergillus flavus* (B) and *Fusarium solani* (C) by *Zingiber officinale* rhizome extrtact at different concentration

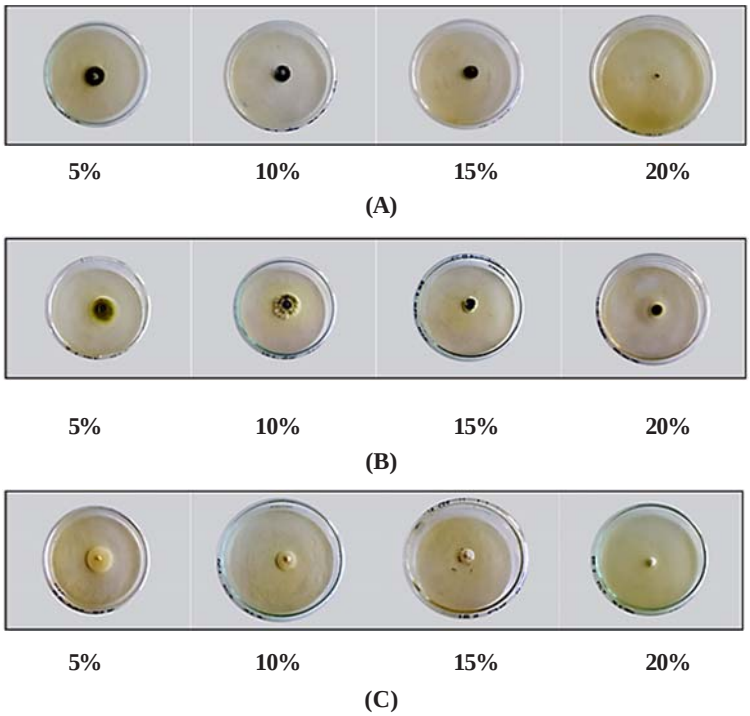

Plate 2. Percentage inhibition of growth of *Alternaria alternata* (A), *Aspergillus flavus* (B) and *Fusarium solani* (C) by *Zingiber officinale +Allium sativum* extrtact at different concentration

with bulb extract of *A. Sativum* showed broad fungitoxic spectrum against a range of fungi tested. The combination of aqueous extract completely inhibited the growth of *Helminthosporium oryzae* Breda de Hann, *Penicillium italicum* Wehmer and *Pythium debarynum* Hesse. In the present study, the two plant extracts has shown antifungal activity under *in vitro* condition and therefore may be recommended further for evaluation of its activity *in vivo* as natural fungicides to control pathogenic fungi in the field, thus reducing the dependence on the synthetic fungicides.

ACKNOWLEDGEMENTS

Authors are thankful to Prof. H. S. Banyal, Chairman, Department of Bio-Sciences, Himachal Pradesh University, Shimla for providing necessary facilities and to the University Grants Commission, New Delhi for providing financial assistance in the form of Dr. D.S. Kothari postdoctoral fellowship.

REFERENCES

1. Cutler, H.G., Cutler, S.J. *Biologically active natural products: Agrochemicals*. CRS Press, Bota Raton: USA, 1999; pp 299.
2. Kumar, A., Srivastava, A., Guleria, S. Bio-efficacy of plant extract against *Alternaria* blight of Rapeseed mustard. *J. Mycol. Pl. Pathol.,* 2006; **36**: 168-70.
3. Raghav, C.S. Medicinal plants on the touchstone of science. *Sci. Rep*.,2003: **40**: 53-60.
4. Mishra, A.K., Dubey, N.K. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *App. and Environ. Microbiol*., 1994; **60**: 1101-05.
5. Pandey, D.K., Chandra, H., Tripathi, V.N. Volatile fungitoxic activity of higher plants with special references to that of *Callistemon lanceolates* D.C. *Phytopathologische-Zeitschrif.*,1982*;* **105**: 175-82.
6. Garber, R.H., Hauston, B.R. An inhibition of *Verticillum albo-atrum* in cotton seed. *Phytopath*., 1959; **49**: 449-56.
7. Thompson, D.P. Fungitoxic activity of essential oil components on food storage fungi. *Mycologia*., 1989; **81**: 151-53.
8. Srivastava, B. Pesticidal activity of some higher plants in management of post harvest bio-deterioration of some food commodities. *Ph.D. Thesis.* Banaras Hindu University, Varanasi-India
9. Vihol, J.B., Patel, K.D., Jaiman, R.K., Patel, N.R. Efficay of plant extracts, biological agents and fungicides against *Alternaria* blight of cumin. *J. Mycol. Pl. Pathol*., 2009; **39**: 516-19.
10. Kumari, L., Shekhawat, K.S., Rai, P.K. Efficacy of fungicides and plant extracts against *Alternaria* blight of Periwinkle (*Catharanthus roseus*). *J. Mycol. Pl. Pathol*., 2006; **36**: 134-37.
11. Dubey, S., Upadhyay, P.D., Tripathi, S.C. Antifungal, physio-chemical and insert repellent activity of essential oil of *Ocimum basilicum*. *Canadian J. of Bot*., 1989; **67**: 2085-87.
12. Varma, J., Tripathi, M., Ram, V.J., Pandey, V.B., Dubey, N.K. Y-asarone, the fungitoxic principle of the essential oil of *Caesulia axillaries. World J. of Microbiol. and Biotech*., 2002; **18**: 277-79.
13. Kumar, R., Dubey, N.K., Tiwari, O.P., Tripathi, Y.B. Essential oil of *Chenopodium ambroisiodes* as botanical fungitoxic against toxigenic strain of *Aspergillus flavus*. In: National seminar on industry and institution interface for new drugs to manage inflammatory diseases. Institute of medical sciences. Banaras Hindu University, Varanasi, India. pp 22.
14. Dubey, N.K., Kishore, N. Fungitoxicity of some higher plants and synergistic activity of their oils. *Trop. Sci.,* 1987; **27**: 23-27.
15. Pandey, V.N., Dubey, N.K. The synergistic activity of volatile fungitoxic compounds from some higher plants. *Acta Botanica Indica.*,1991; **19**: 290-95.