Available online through

www.jbsoweb.com

Research Article

IN VITRO SCREENING OF NATURAL PRODUCTS ON *FUSARIUM OXYSPORUM* FROM ROTS IN GINGER

Chaithra M, Shobha K.S*, Pallavi S, Sachidananda Swamy H.C, Vivek M.N, Manasa M, Yashoda Kambar and Asha M.M

P.G. Department of studies and Research in Microbiology Sahyadri Science College (Autonomous), Shimoga, Karnataka, India

***Correspondence**

Shobha K.S

P.G. Department of studies and Research in Microbiology Sahyadri Science College (Autonomous), Shimoga, Karnataka, India

DOI: 10.7897/2321-6328.01309

Article Received on: 09/07/13

Accepted on: 20/09/13

Abstract

Inhibitory potential of natural products on pathogenic *Fusarium oxysporum* isolated from rhizome rots in ginger prevalent in Shimoga district, Karnataka, India was carried out in the present work. Crude and concentrated cow urine, aqueous extracts of leaf and flowers of *Parthenium hysterophorus* and petroleum ether extract of latex of *Calotropis procera* were tested by Poison food technique. Antifungal potential was assessed by measuring the mycelial diameter in test and control plates. Concentrated cow urine and aqueous extracts of leaves of *Parthenium hysterophorus* were best in inhibiting, with mycelial diameters ranging between 15-32 mm when compared to control plates (20-35 mm). Latex extract was moderate in inhibition while flower extract of *Parthenium hysterophorus* was least effective.

Keywords: Natural products, Antifungal potential, Ginger rots, Poison food technique.

INTRODUCTION

Zingiber officinale Rosc. (Ginger) belonging to the family Zingiberaceae (Hayden *et al*, 2004) is an important commercial crop grown for its aromatic rhizomes which are used as a spice and a medicine (Kavyashree, 2011). It is an important crop that earns a sizeable amount of foreign exchange for the country (Tarafdar and Saha, 2007). India is the largest producer of ginger accounting for about 1/3rd of total world output. Ginger is grown in various states such as Kerala, Karnataka, West Bengal, Andhra Pradesh, Orissa, Arunachal Pradesh and Sikkim, India (Sharma *et al*, 2010). The production of ginger, however, is largely affected by diseases caused by bacteria, fungi, viruses, mycoplasma and nematodes. The crop suffers from diseases like bacterial wilt caused by *Ralstonia solanacearum* rhizome rot caused by *Pythium* (Stirling *et al*, 2009) and *Fusarium* species (Paret *et al*, 2010; Senapati and Ghose, 2005; Dake and Edison, 1989). India is the leading producer of ginger in the World. Over the last few years rhizome diseases have affected the many states of India resulting in decline of rhizome yield. It is estimated that more than fifteen fungi are responsible for rot in ginger which can be controlled by applying synthetic chemical fungicides (Dohroo, 1993; Kumar, 1977). As these synthetic chemicals have some adverse effect on environment and human health due to non judicious and inappropriate application the search for an alternative from natural sources is an urgent need of the study. The present study was designed to investigate inhibitory effect of natural products against *Fusarium oxysporum* f. sp. Zingiberi isolated from rhizome rot specimen of ginger.

MATERIALS AND METHODS

Sampling

Infected rhizome and soil samples were collected from crop fields of Ripponpet, in Shimoga district, India. Both the samples were preserved in aseptic condition until further processing.

Isolation of *Fusarium* species

Rotten portions of Rhizomes of ginger were surface sterilised in 0.1 % mercuric chloride for one minute and 1-1.5 cm pieces were cut from the rhizomes. About four to five pieces were inoculated on Potato Dextrose Agar (PDA) plates incorporated with antibacterial antibiotic like Streptomycin (300 mg/litre) by direct plating method. Infected soil samples were serially diluted in physiological saline up to 10^{-7} dilutions and spread plated on PDA plates incorporated with antibacterial antibiotics like Streptomycin (300 mg/litre). Both the sets of plates were incubated a 25-27^{o}C for 48-72 hours and observed for the appearance of fungal pathogen.

Characterization of *Fusarium* species

The Fungal colonies developed were observed for their mycelia colour and pigmentation in background of the mycelium on the media selected for isolation (Shanmugam *et al*, 2013). The identification of genus was done by wet mounting of the sporulated mycelium microscopically in 45 X objective. Confirmation of genus was done by noting down the type and arrangement of spores on mycelium, by referring standard literatures (Barnet and Hunter, 1972).

Preparation of natural products

The natural products selected for antifungal assay were

- Cow urine sample from local breed of Honnali taluk, Davangere district, India. Sample was divided into two halves, first half was refrigerated as crude sample and other was subjected for concentration by keeping it in Oven at 50^{o} C for four days to get concentrated cow urine sample (Shalini *et al*, 2007).
- Two plant materials like *Parthenium hysterophorus* and *Calotropis procera* were selected. 10 % leaf and flower

extracts of parthenium were prepared in sterile water. Incase of calotropis 10 % Petroleum ether extract of Latex was prepared.

***In-vitro* Antifungal assay**

Antifungal potential of above mentioned extracts was tested on isolated fungal pathogen by poison food technique. One ml of each sample was transferred into sterile Petri plates, mixed with 20 ml of PDA media and allowed for solidification. For each natural product one plate without test sample was kept as control. 8 mm mycelia disc of actively growing fungal colony was transferred into all the Petri plates and incubated at 26^0C for 48 to 72 hours. After incubation the mycelia; diameter was measured in both test and control plates to assess the inhibitory potential (Gupta and Tripathi, 2011).

RESULTS

Both the rhizome and soil samples yielded positive results by the appearance of cottony white mycelial growth on the plates (Figure 1). The cream coloured Colonies with a slight pink pigmentation On PDA were subjected for microscopic characterization. The colonies were identified as *Fusarium* species based on the spores produced. The species was confirmed as *F. oxysporum* based on size and appearence of conidia by referring standard literature. Macroconidia are slender and of medium length. Microconidia were small, 1–3 septate and are formed in false heads on very long phialides or branched conidiophores (Figure 2). The inhibition potential of natural products like concentrated cow urine sample, petroleum ether extract of milky latex of *Calotropis procera* and flower and leaf extract of parthenium hysterophorus tested on *F. oxysporum* yielded variable results. Leaf extract of parthenium and concentrated cow urine were best in inhibiting the fungal pathogen. Flower extracts was least effective on the isolate. Moderate result was exhibited by crude cow urine and *Calotropis procera* (Figure 3). Inhibitory effect of fungicides on the fungal isolate was also done by the same poison food technique. Fungicide sten-50 and Sahara were best when compared with controle. Mancozeb was ineffective on the isolate, while moderate result was shown by lime and copper sulphate. Combination of natural products like cow urine with the effective fungicide may give enhanced inhibition of the pathogen.

Table 1: Inhibitory Efficacy of Natural Products on *F. oxysporum*

Sample	Mycelial diameter in mm after 72 hours of incubation			
	Control	T-1	T-2	Mean
S1	27	15	25	20
S2	35	34	30	32
S3	23	18	17	17
S4	23	15	16	15

S1- Concentrated cow urine; S2- Calotropis in Pet-ether (1:10dilution); S3- Parthenium flower crude extract; S4- Parthenium leaf crude extract; T1- test plate1; T2-test plate 2

Table 2: Inhibitory Efficacy of Synthetic compounds on *F. oxysporum*

Sample	Colony diameter in cm after 72 hours of incubation			
	Control	T-1	T-2	Mean
S5	3.5	2.4	2.7	2.5
S6	1.9	2.3	2.2	2.2
S7	1.5	1.5	1.5	1.5
S8	1.5	0.0	0.0	0.0
S9	1.5	0.0	0.0	0.0

S5- Lime (1:5dilution in sterile water); S6- Copper sulphate+ Calcium carbonate; S7- Mancozeb; S8- Sahar; S9-Sten-50

Figure 1: Colony of *F. oxysporum*

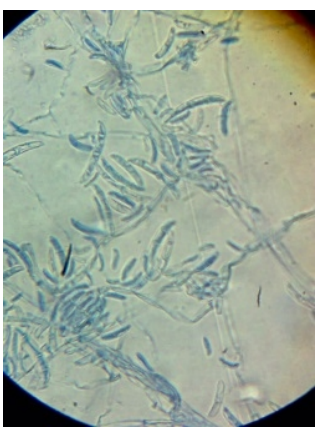

Figure 2: Microscopic view *F. oxysporum*

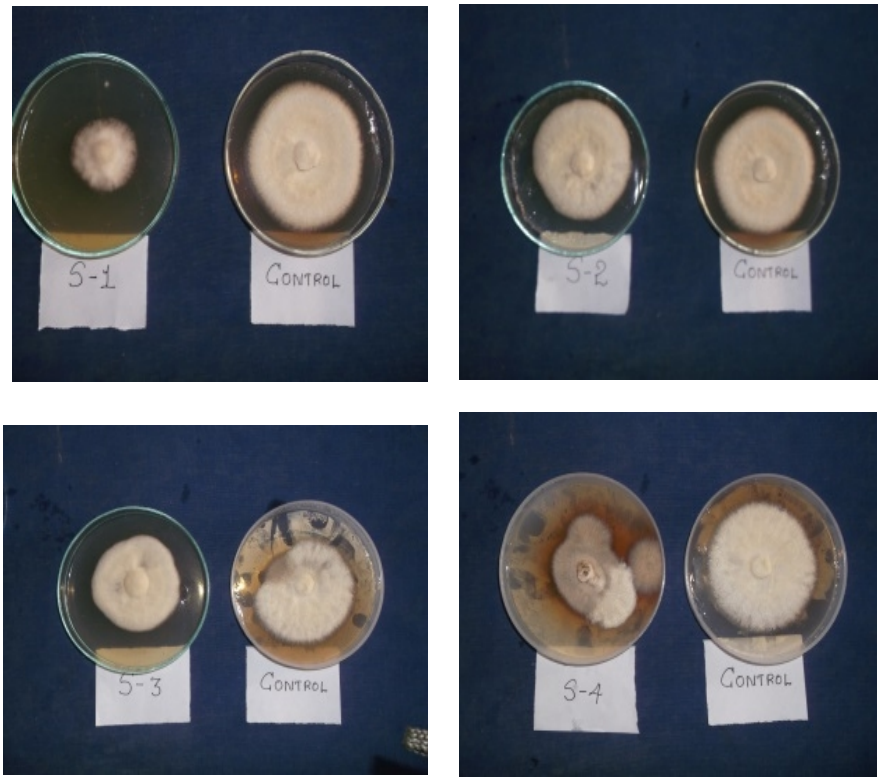

Figure 3: Inhibitory Efficacy of Natural products on *F. oxysporum*

DISCUSSION

Studies on ginger rots by fungal pathogen were carried out by many workers throughout India (Kumar, 1977; Dake and Edison, 1989). Pathogens like *Phytopthora*, *fusarium* sp. (Yang *et al*, 1989; Senapati and Ghose, 2005) were the most common fungi responsible for drastic reduction in yield of ginger crops in Southern india (Poudyal *et al*, 2012). Crop loss due to rot-causing fungal pathogens is a significant problem. The most common method of control is the use of chemical fungicides (Meenu and Dohroo, 2009) However, environmental concerns, costs, development of resistance in pathogens increased interest in alternatives such as plant extracts, antagonistic microbes and others to traditional synthetic chemical fungicides (Sealy *et al*, 2007; Ramteke and Kamble 2011). The present work concentrated on finding out the effect of natural products, which are eco-friendly and less harmful than commercial synthetic compounds like fungicides. Tripathi (2011) showed fungi toxic activity of *Solanum torvum* against *Fusarium sacchari*. Poisoned food technique has been routinely employed to screen the effect of plants and their compounds against fungi. The antifungal activity is observed as reduction in the mycelial growth of fungus in poisoned plates when compared to control plates. It has been employed by several researchers to evaluate antifungal activity of plants (Nunez *et al*, 2010; Gupta and Tripathi, 2011). In malnad region Ginger crop is severely affected by rots of *Fusarium* sp. which was not controlled even by the best biological control measures such as Trichoderma and Neem cake applications. So the present work was initiative of finding alternative remedy which was economical and eco-friendly. It had given positive results in the crude extracts itself. Significant finding was the concentrated cow urine which is available as a very cheap source for the growers. As the present work was an *in vitro* study, further work is going on rhizome treatment with concentrated cow urine sample and parthenium leaf extracts before sowing. Even the application of these to the crop fields may even give better results. Exploitation of compounds from natural products which retards the growth of undesirable organisms would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides with special reference to management of plant diseases.

ACKNOWLEDGEMENTS

Authors express sincere thanks to the Principal, Sahyadri Science College (Aut.) for providing facilities to carry out work.

REFERENCES

1. Barnett HL and Hunter BB. Illustrated Genera of Imperfect Fungi, Burgess Publishing Co. Minneapolis, Mn, USA; 1972.
2. Dake GN, Edison S. Association of pathogens with rhizome rot of ginger in Kerala. Indian Phytopathology 1989; 42(1): 116-119.
3. Dohroo NP. ICAR report on Multi location project on rhizome rot of ginger. Dr YS Parmar University of Horticulture and Forestry, Nauni-Solan 1993; 4(4): 38.
4. Gupta SK, Tripathi SC. Fungi toxic activity of *Solanum torvum* against *Fusarium sacchari*. Plant Protection Science 2011; 47(3): 83-91.
5. Hayden AL, Brigham LA, Giacomelli GA. Aeroponic Cultivation of Ginger (*Zingiber officinale*) Rhizomes. ISHS Acta Horticulturae 2004; 659(2): 397-402.
6. Kavyashree R. An efficient *in vitro* protocol for clonal multiplication of Ginger- var. Varada. Indian Journal of Biotechnology 2009; 8: 328-331.
7. Kumar SNS. Ginger rot by *Fusarium solani*. Journal of Plant Crops 1977; 5: 122.
8. Meenu G and Dohroo NP. Magnitude of Rhizome rots pathogens of ginger in Himachal Pradesh. Plant Disease Research. 2009; 24(1): 82.
9. Nunez YO, Salabarria S, Collado IG, Hernandez Galan R. Antifungal activity of extracts and terpene constituents of aerial parts of Juniperus lucayana. The Revista Latinoamericana de Química 2010; 38(3): 145-152.
10. Paret ML, Cabos R, Kratky BA, Alvarez AM. Effect of plant essential oils on *Ralstonia solanacearum* Race 4 and bacterial wilt of edible

ginger. Plant Disease 2010; 94(5): 521-527. http://dx.doi.org/10.1094/PDIS-94-5-0521
11. Poudyal BK. Jeevatu: One of the Best Bio-Agents for the Control of Soft Rot of Ginger. International Conference on Environment Science and Biotechnology 2012; 48(13): 66-70.
12. Ramteke PK, Kamble SS. Evaluation of phyto extracts against *Fusarium solani* (Mart.) Sacc. Causing rhizome rot of ginger (*Zingiber officinale* Rosc.). Current Biotica 2011; 4(4): 469-474.
13. Sealy R, Evans MR, Rothrock C. The effect of garlic extracts and root substrate on soil borne fungal pathogens. Hor. Technology 2007; 17(2): 169-173.
14. Senapati AK, Ghose S. Screening of ginger varieties against rhizome rot disease complex in eastern ghat high land zone of Orissa. Indian Phytopathology 2005; 58(4): 437-439.
15. Shalini D, Sagar SD, Kulkarni S and Hegde YR. Management of rhizome rot of ginger by botanicals. Internat. J. Plant Sci 2007; 2(2): 155-158.
16. Shanmugam V, Thakur H, Kaur J, Gupta S, Rajkumar S, and Dohroo NP. Genetic diversity of *Fusarium* sp. inciting rhizome rot of ginger and its management by PGPR consortium in the western Himalayas. Biological control 2013; 66(1): 1-7. http://dx.doi.org/10.1016/j.biocontrol.2013.03.001
17. Sharma BR, Dutta S, Roy S, Debnath A, Roy MD. The effect of soil physico-chemical properties on rhizome rot and wilt disease complex incidence of ginger under hill agro-climatic region of West Bengal. Plant Pathology Journal 2010; 26(2): 198-202. http://dx.doi.org/10.5423/PPJ.2010.26.2.198
18. Stirling GR, Turaganivalu U, Stirling AM, Lomavatu MF and Smith MK. Rhizome rot of ginger (*Zingiber officinale*) caused by *Pythium myriotylum* in Fiji and Australia. Australasian Plant Pathology 2009; 38: 453–460. http://dx.doi.org/10.1071/AP09023
19. Tarafdar J, Saha N. Correlation study on population dynamics of ginger soft rot inciting pathogens under different organic amendments, disease incidence and its survival in Darjeeling hill soils. Proceedings of the 13th ISTRC Symposium; 2007. p. 165-169.
20. Yang KD, Kim HM, Lee WH and So IY. Studies on rhizome rot of ginger caused by *Fusarium oxysporum* f. sp. zingiberi and *Pythium zingiberum*. Korean Journal of Plant Pathology 1988; 4(4): 271-277.

Cite this article as:
Chaithra M, Shobha K.S, Pallavi S, Sachidananda Swamy H.C, Vivek M.N, Manasa M, Yashoda Kambar and Asha M.M. In vitro screening of natural products on Fusarium oxysporum from rots in ginger. J Biol Sci Opin 2013;1(3):182-185 http://dx.doi.org/10.7897/2321–6328.01309

Source of support: Nil; Conflict of interest: None Declared