Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2022, 12(6)

ISSN : 2249-7412
CODEN (USA): AJPSKY

In vitro Evaluation of Botanicals against Red Rot of Sugarcane (*Colletotrichum falcatum*)

Biraj Poudel*, Archana Bhatt and Sandip Panth

Department of Agriculture, Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur, Nepal

***Corresponding author:** Biraj Poudel, *Department of Agriculture, Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur, Nepal* , E-mail: poudelbiraj85@gmail.com

Received date: May 11, 2022, Manuscript No. AJPSKY-22-13620; **Editor Assigned date:** May 13, 2022, PreQC No. AJPSKY-22-13620 (PQ); **Reviewed date:** May 24, 2022, QC No. AJPSKY-22-13620; **Revised date:** June 3, 2022, Manuscript No. AJPSKY-22-13620 (R); **Published date:** June 10, 2022, DOI: 10.36648/2249-7412.12.6.219

Citation: Poudel B, Bhatt A, Panth S (2022) In vitro Evaluation of Botanicals against Red Rot of Sugarcane (*Colletotrichum falcatum*). Asian J Plant Sci ResVol.12 No.6: 219

ABSTRACT

Red rot of sugarcane caused by Colletotrichum falcatum is one of the major economically important diseases of sugarcane causing significant reduction in yield quantity and quality. Synthetic fungicides are used to control the fungi usually but are harmful for human health and environment. Keeping in view the potential of some bioactive indigenous plant, a study was carried out to study on the efficacy against this fungi. The present experiment was conducted to evaluate the effectiveness of five botanical extracts, Azadirachta indica; Neem, Datura stramonium; Datura, Nicotinia tabaccum; Tobacco, Justicia adhatoda; Asuro, Allium sativum; Garlic at two concentration 25% and 50% against Colletotrichum falcatum in vitro using poisoned food technique in completely randomized design. All the tested botanical extracts significantly inhibited the mycelial growth of this pathogen as compared to control. After 18 days, among the plant extracts 50% Azadirachta indica showed maximum inhibitory effect (72.79%) along with its lower concentration 25% (67.93%) followed by 50% Datura stramonium (69.58%) and its lower concentration 25% (65.72%). Similarly lowest inhibitory effect was shown by 25% Tobacco (7.630%) followed by 25% Garlic (28.86%). The study indicated that botanicals such as Neem, Daturo, Asuro showed better performance even at its lower concentration thus, such effective botanicals could be used at appropriate concentration to control red rot of sugarcane. Result of this study indicates that use of botanicals for control of Colletotrichum falcatum is safe and is a good alternative to synthetic chemical fungicides.

Keywords: *Botanicals; Colletotrichum falcatum; Indigenous; Poisoned food; Sugarcane*

Introduction

Sugarcane (*Saccharum officinarum L.*) is a major cash crop which is cultivated in many parts of the world mostly in the tropics (3580 N, 3580 S) [1]. Sugarcane is one of the most important commercial crops of Nepal. It is cultivated globally to produce sucrose, ethanol, and other by-products. Globally, sugarcane is an important source of commercial sugar accounting for almost two thirds of global sugar production [2]. More than 100 diverse diseases have been reported in sugarcane which is caused by fungi, viruses, bacteria, nematodes and phytoplasma [3]. Among all this disease red rot is in topmost concern. The red rot also has constituted one of the most important limiting factors in the growth and release of certain desirable cane varieties [4]. Red rot disease was first reported in Java (now Indonesia, in 1893) and epidemics have been common in India ever since it was first reported there [5]. Sugarcane production sometimes declined due to various stresses, pests, and diseases. The worldwide loss in cane yield and sugar recovery is approximately 5%-10% per annum. Usually, commercial fungicides are being used for the control of red rot disease which is much harmful for human and agro ecosystem. There has been a rising concern on the research plant extracts for control of pest and diseases in agriculture which are not as much of harmful to the human health and environment [6]. Since 1893 the casual organism of the red rot of sugarcane disease has been considered as being *Colletotrichum falcatum* went a one of the fungi imperfect. *Colletotrichum falcatum* hydrolyses the stored sucrose in sugarcane

set by producing the enzyme invertase which breaks the sucrose molecule into glucose and fructose resulting into increased molasses [7]. The perfect stage of the red rot fungus has been found occurring very sufficiently under natural field conditions on fading and dead leaves and occasionally on dead young top portions of stems of sugarcane-in *Louisiana*. Several important cultivated varieties, like, Co419, CoC671, CoC86062, and CoC92061, have been removed from cultivation because of their high susceptibility to red rot disease [8]. Red rot infection of sugarcane reduces yield as well as juice qualities, such as brix value, sucrose content, and purity [9]. Red rot infection reportedly reduced sucrose content by 75% [10]. Decrease in juice content and quality results greater losses for both cane grower and sugar factories. Generally commercial fungicides are being used for the control of red rot disease which is harmful for human and agro-ecosystem [11]. It is difficult to control red rot disease through chemotherapy (fungicides/ chemicals) because impervious nature of rinds and fibrous nodes at cut ends do not allow sufficient absorption in setts [12]. Plants contain extensive variety of secondary metabolites, such as *tannins*, *terpenoids*, *alkaloids*, and *flavonoids*, having antimicrobial properties [13]. Growth inhibition of *C. falcatum* was observed by various plant extracts. Use of some plant extracts such as the seeds of *Piper nigrum* (Black Pepper), *Rhizomes* of *Zingiber officinale* (Ginger), leaves of *Azadirachta indica* (Neem), leaves of *Carica papaya* (Papaya) and leaves of *Nicotiana tabacum* (Tobacco) in the suppression of *Colletotrichum sp*. The efficacy of plant extracts of *Allium sativum* and *Azadirachta* indica against *Colletotrichum* spp has been expressed by Kumar and Yadava. Neem as a bio control agent used for centuries in Asia as a potential antifungal agent [14].

In this regard effect of extracts of indigenous five Plants Neem (*Azadirachta indica*), Tobacco (*Nicotiana tabacum*), Dhaturo (*Datura stramonium*), Garlic (*allium sativum*), and Asuro (*Justica adhatoda*) were tested in two concentration (25% and 50%) against *Colletotrichum falcatum* to evaluate inhibitory effect on the growth of the fungi in *vitro*.

Materials and Methods

The research was conducted on laboratory of plant pathology at *Gokuleshwor* agriculture and animal science college located at 29039’38.5” N and 80032’45.8” E, *Sudurpaschim* Province in completely randomized design using poisoned food technique for botanicals screening [15,16]. Five botanical leaf extracs (*Azadirachta indica*; Neem, *Datura stramonium*; *Dhatura, Nicotinia tabaccum*; Tobacco, *Justicia adhatoda*; *Asuro*, *Allium sativum*; Garlic) were evaluated in two different concentrations *viz.* 25% and 50% respectively. Each treatment was replicated four time.

Isolation and maintenance of pure culture

The pathogen (*Colletotrichum falcatum*) was isolated from the disease sample collected from periphery of GAASC, *Baitadi*. The spores were teased from infected portion for microscopic inspection to check the presence of pathogenic fungus. After confirmation the presence of fungus, the diseased sample were chopped using sterilized blades into 4 mm-6 mm size and then disinfected by dipping in 0.5% Sodium hypochlorite solution for 2 minutes followed by 3 rinses with the distilled water. The cut sample was dried on sterilized bloating paper. After this, sterilized samples were placed in the Potato Dextrose Agar (PDA) medium inside *Laminar* air flow using sterilized forceps. Petri plates were sealed using parafilm and incubated in bacteriological incubator for 18 days in 28°C ± 10°C temperature. The mor-phological characteristics of conidia was verified in accordance with the morphological characters described by [17]. Culture was purified by transferring small pieces of agar containing spores to another petri plate containing fresh PDA media and incubated at 28°C ± 10°C for 20 days. This Pathogen was sub-cultured three times to obtain pure culture.

***In vitro* evaluation of botanical extracts**

Leaves of selected plant species were collected and washed with running tap water. Leaves were crushed in mixture/ grinder (1:1 w/v) as per Thaware et al. [16]. Thus, obtained extracts were filtered using double layered muslin cloth and whatman’s filter paper. The solution was then filtered using *Whatman’s* filter paper No. 1. Thus, obtained solution was considered stock/standard solution 100%. Now the treatment solution was adjusted.

The PDA media was prepared as per requirement for 25% and 50% solution. This PDA was sterilized in autoclave. The prepared solution was allowed to cool (40°C) and pinch of streptomycin sulphate (0.25 gm/ltr) was added to check the bacterial growth. The plant extract prepared was added to media to obtain final standard solution of 25% and 50% using the food poisoned technique by Nene and Thapliyal; Thaware et al. [15,16]. 25 ml of amended media was poured in each 90 mm sterilized petri plates and allowed to solidify. Control treatment was prepared without

adding plant extracts. A circular disc of 5 mm diameter from 16 days old culture of *Colletotrichum falcatum* was cut using sterilized cork borer and inoculated at the center of solidified amended as well as control plates. These plates were then incubated in incubator for growth at 27°C ± 10°C temperature. Record of the growth was measured in every 12 hours interval until the control plate achieves complete growth.

Growth inhibition test and statistical analysis

The observation on mycelial growth was recorded every 12 hrs after inoculation *i.e.* at 6 AM and 6 PM for 18 days using a scale. The percentage inhibition of mycelial growth over control was calculated using the following formula [18]:

PGI= ((C-T))/C x 100

Where,

PGI: Percentage Growth Inhibition,

C: Growth of hyphae of *Colletotrichum falcatum* in control (cm) and

T: Growth of hyphae of *Colletotrichum falcatum* in treatment (cm)

Recorded data were entered and processed using Microsoft Excel (2021) and analysis of variance was done using M-Statc. Mean comparison was done using Least Significance Difference (LSD) test at 0.05 level of significance.

Result and Discussion

Five different botanical extracts were evaluated in two concentration 25% and 50% for their efficacy against *Colletotrichum falcatum in vitro*. The result (Table 1) exposed that all the tested botanicals inhibited the growth of pathogen over untreated control. Different plant extract displayed different level of fungicidal behavior against test fungi. Significant difference ($P \leq 0.00$) was attained among different extract in their inhibition effect. Increase in effectiveness was detected with increase in concentration. After 2 days of inoculation maximum growth inhibition was recorded in Garlic 50% (44.94%) and Dhaturo 25% and 50% (38.20%) followed by Asuro 50% (37.08%) which were at par with Garlic 25% (33.71%). Minimum inhibition was obtained in Tobacco 50% (1.685%) followed by Tobacco 25% (8.427%) and Neem 50% (26.41%). Similarly, after 4 days of inoculation Garlic 50% (59.04%) showed maximum inhibition, Dhaturo 50% (58.36%) followed by Garlic 25% (44.71%) and Neem 50% and 25% (41.98% and 40.62%) followed by Dhaturo 25% (41.64%) whereas, Tobacco 25%, 50% (9.215% and 10.58%) and Asuro 25%, 50% (37.89% &39.35%) recorded least inhibition percentage. After 6 days of inoculation, maximum growth inhibition was recorded in Garlic 50% (55.67%) and Dhaturo 50% (55.14%) followed by Neem 50% (46.49%). Whereas minimum inhibition was obtained in Tobacco 25% and 50% (8.108% and 11.89%) followed by Garlic 25% (36.76%). After 8 days of inoculation maximum growth inhibition was recorded in Neem 50% (58.51%) and Dhaturo 50% (56.22%) followed by Neem 25% (55.26%). Hereby, minimum inhibition was recorded in Tobacco 25%, 50% (-1.913% and 23.90%) followed by Garlic 25% (28.30%). Similarly, after 10 days, Neem 50%, 25% (63.17% & 58.85%) ranks 1st, Dhaturo 50%, 25% (61.68 and 52.61%) followed by Asuro 50%, 25% (52.08% and 47.92%), Garlic 50%, 25% (40.99% and 20.65%) finally the least inhibition was showed by Tobacco 25% and 50% (-7.860% and 34.83). In 12th day after inoculation, maximum inhibition was observed in Neem 50%, 25% (63.60% and 55.73%) followed by Dhaturo 50%, 25% (60.20% and 55.87%) and Asuro 50%, 25% (54.80% and 43.33%). Whereas minimum inhibition was observed in Tobacco 25% (-5.068%) and Garlic 25% (14.0%). After 14 days of inoculation maximum inhibition was observed in Neem 50% (67.83%) followed by Dhaturo 50% (66.14%) in contrast minimum inhibition was observed in Tobacco 25% (1.278%) and Garlic 25% (19.63%). In day 16, maximum inhibition was observed in Neem 50% (70.61%) which is at par with Dhaturo 50% (68.40%) followed by Neem 25% (65.10%) which is at par with Asuro 50% (58.54%) whereas minimum inhibition was shown by tobacco 25% (3.470%) followed by garlic 25% (26.64%). After 18 days, inhibition percentage varied from 7.630% to 72.79%. Neem proved to be most effective botanical extract in both concentration (*i.e.* 50% and 25%) showing inhibition of 72.79% and 67.93%. This was followed by all concentration (50% and 25%) of Dhatura (69.58% and 65.72%) and 50% Asuro (64.34%). (25% and 50%) Neem (67.93% and 72.79%) and 50% Dhatura (69.58%) were significantly indifferent with each other. 25% Tobacco (7.630%) was least effective in restricting the mycelial growth followed by 25% Garlic (28.86%) and 50% Garlic (44.49%).

S.N.	Botanical	Conc.	Percentage growth inhibition (%)								
		(%)	Day 2	Day 4	Day 6	Day 8	Day10	Day12	Day14	Day16	Day18
1	Neem	25	29.21cd	40.62b	43.51bcd	55.26ab	54.85bc	55.73b	60.98b	65.10bc	67.93abc
		50	26.41d	41.98b	46.49b	58.51a	63.17a	63.60a	67.83a	70.61a	72.79a
2	Dhaturo	25	38.20b	41.64b	45.13bc	49.90bc	52.61c	55.87b	59.70b	62.55c	65.72bc
		50	38.20b	58.36a	55.14a	56.22ab	61.48ab	63.20a	66.14a	68.40ab	69.58ab
3	Tobacco	25	8.427e	9.215c	8.108e	-1.913e	-7.860g	-5.068f	1.278g	3.470h	7.630g
		50	1.685f	10.58c	11.89e	23.90d	34.83e	35.73d	39.49d	44.59e	48.35e
4	Garlic	25	33.71bc	44.71b	36.76d	28.30d	20.65f	14.0e	19.63f	26.64g	28.86f
		50	44.94a	59.04a	55.67a	51.24abc	40.99e	30.67d	34.38e	39.80f	44.49e
5	Asuro	25	27.53cd	37.89b	38.38bcd	47.61c	47.92cd	43.33c	47.39c	51.43d	54.04d
		50	37.08b	39.25b	37.83cd	44.93c	52.08c	54.80b	58.54b	61.74c	64.34c
	SEM (±)		2.17	2.67	2.57	2.99	3.38	3.48	3.37	3.28	3.2
	CV %		15.21	15.09	15.01	12.43	12.63	9.43	7.49	6.32	6.66
	LSD at 5%		6.295	8.354	8.214	7.434	7.675	5.608	4.923	4.468	5.037

Table 1: Efficacy of different botanical extract on growth of *C. falcatum in vitro*

Fungi-toxicity of plant extract might be due to antifungal metabolite present in plant. Variation in antifungal activity of different plant extract is due to variation in the content of active chemicals in plant extract [19]. Neem leaves possess azadirachtin which contain antifungal properties. Irum found that aqueous extract of plant species *i.e.* Dhatura metal, *Azadirachta indica*, *Parthenium hysterophorus* and *Ocimum* sanctum were tested *in vitro* [20]. Among them *A. indica* and *D. metal* inhibited *mycelial* growth of *F. oxysporium*, *F. sp. ciceri* supporting our present study. Our result was in accordance with Abbas et al. who reported maximum growth suppression by *Azadiracta indica* (89.9%) followed by *Dhatura stramonium* and *Allium sativum* [21]. Also, our results are in accordance with who reported leaf extract of *Azadirachda indica*, *D. stramonium* having fungitoxic effect against *A. brassicola*, *Colletotrichum capsci*, *F. oxysporum*, *R. solani and S. sclerotiorum* [22]. Similar observations were expressed by Ahmed et al. (2002) who found the efficacy of A. indica against *Bipolaris oryzae* under *in vitro* condition [23]. Sharma and Tamta showed that leaf extract of Curcuma domestica and Dhatura metal inhibited the conidial as well as mycelial growth of *Colletotrichum spp* [24].

Our research result is in line with Bernardo-Mazariegos et al. who recorded that *Justicia spp* consists of Silver Nano Particle (AGNP) which have significant mycelial growth inhibitory effect on M. phaseolina (79.6%), A. alternate (60.10%) while in *Colletotrichum spp* and F. solani it shows lower mycelial growth inhibition 40% and 30% respectively after 9 days of inoculation [25].

Osmotin protein isolated from protein can inhibit *in vitro*, the growth of number of unrelated pathogens, a survey of 31 isolate representing 18 fungus genera indicated that sensitivity determined at the genus level. The growth of Bipolaris fusarium and Phytopthera species was very sensitive whereas A. flavus, R. solani, *Macrophomina* spp. are highly resistance to osmotin while Phytopthera and Colletotrichum appears to be moderately sensitive as a genus which Aspergillus seems to be insensitive [26]. Methanol extract of tobacco leaf produce zone of inhibition of 9.5 mm against Candida whereas the water extract of tobacco has no inhibitory activity on fungi Candida [27]. The plant extract of *Nicotinia tabacum* showed no growth inhibition difference of mycelia of *Colletotricum spp* [28].

Methanol and acetone extract of *Allium sativum* inhibit the *mycelial* growth of *Colletotrichum falcatum* whereas aqueous garlic extract showed statistically inferior result as compared to acetone, methanol, and kerosene solution. The aqueous solution of *Allium sativum* suppressed the growth of *C. falcatum* as 53.43%, 47.43%, 42.43%, and 37.43% after 4^{th}, 6^{th}, 8^{th}, and 10^{th} day respectively which is in line with our research [29].

Conclusion

Colletotrichum falcatum is a worldwide disease of economic importance in sugarcane production. It causes severe loss in yield, quality, and quantity. Although different chemicals fungicides are commercially available in market to control this pathogen, their indiscriminate application result in several health hazard, and environmental impact. To address this botanical extract can be the suitable alternative. In this experiment Neem (Azadirachta indica) and Dhaturo (Dhatura stramonium) exhibited higher inhibition percentage. These botanical extracts possess a potential ability

to be used as novel fungicides alternative to harmful chemical as they give minimum environmental impact and health hazard to consumer in contrast to synthetic chemical fungicides. Generally, the exploration of these botanicals could be safe, ecofriendly, and cost-effective approach for the management of the pathogen. Our present research finding is confined to laboratory conditions so; more *in vitro* and field trial are required in future to validate this finding.

Acknowledgement

Authors are thankful to Mr. Rajan Poudel, Asst. Professor, Institute of agriculture and animal science, Paklihawa Campus for his continuous support and guidance. Gokuleshwor agriculture and animal science college for providing required materials and equipment.

References

1. Jayakumar V, Bhaskaran R, Tsushima S (2007) Potential of plant extracts in combination with bacterial antagonist treatment as biocontrol agent of red rot of sugarcane. Can J Microbiol 53: 196-206.
2. Menossi M, Silva-Filho MC, Vincentz M, Van-Sluys MA, Souza GM (2008) Sugarcane functional genomics: Gene discovery for agronomic trait development. Int J Plant Genomics 11: 1-11.
3. Bharti YP, Vishwakarma SK, Kumar A, Singh A, Sharma ML, et al. (2012) Physiological and pathological aspects of some new isolates of *colletotrichum falcatum* causing red rot disease in Saccharum spp complex. Acta Phytopathol Entomol Hung 47: 35-50.
4. Carvajal F (1943) The perfect stage of *colletotrichum falcatum*.
5. Barber CA (1901) Sugarcane disease in Godawari and Ganjam districts. Madras Department of Agriculture Bulletin 2: 181-193.
6. Costa TR, Fernandes FL, Santos SC, Oliveira MA, Liao LM, et al. (2000) Antifungal activity of volatile constituents of eugenia dysenterica leaf oil. J Ethnopharmacol 72: 111-117.
7. Sehtiya HL, Phawan AK, Virk KS, Dendsay J (1993) Carbohydrate metabolism in relation to *Colletotrichum falcatum* in resistant and susceptible sugarcane cultivars. Indian Phytopathol 46: 83-85.
8. Viswanathan R, Samiyappan R (2001) Role of chitinases in Pseudomonas spp. induced systemic resistance against *Colletotrichum falcatum* in sugarcane. Indian Phytopathol 54: 418-423.
9. Kalaimani T (2000) Biological control of red rot sugar cane caused by (*Colletrotrichum falcatum* Went). Indian Sugar 50: 489-492.
10. Viswanathan R, Samiyappan R (1999) Red rot disease in sugarcane: A major constraint for the Indian sugar industry. Sugar Cane pp: 9-15.
11. Tariq SA, Pervez A, Ali R, Zafar H (2017) Antifungal activity of some plant extracts *in vitro* against *colletotrichum falcatum* causing red rot of sugarcane. Int J Biol Biotech 14: 581-584.
12. Agnihotri VP (1990) Disease of sugarcane and sugarbeet. Oxford and IBH Publishing Co Pvt. Ltd, India
13. Cowan MM (1999) Plant products as antimicrobial agents. Clin microbiol Rev 12: 564-582.
14. Chaturvedi R, Razdan MK, Bhojwani SS (2003) Production of haploids of neem (Azadirachta indica A. Juss.) by anther culture. Plant Cell Rep 21:531-537.
15. Nene YL, Thapliyal PN (1979) Fungicides in plant disease control. New Delhi: Oxford and JBH Publishing Co.
16. Thaware DS, Furgo PA, Magar SV, Jadhav YT, Karande RA (2010) *In vitro* evaluation of different fungicides, plant extracts and bio-agents against alternaria alternate (Fr.) keissler causing leaf blight of cowpea. Int J Plant Prot 3: 356–360.
17. Sutton BC (1992) The genus Glomerella and its *anamorph Colletotrichum*. *Colletotrichum*: Biology, Pathology and Control pp: 1-26.
18. Vincent JM (1947) Distribution of fungal hyphae in the presence of certain inhibitors. Nature 159: 850-850.
19. Shrestha AK, Tiwari RD (2009) Antifungal activity of crude extracts of some medicinal plants against fusarium solani (Mart.) Sacc. ECOPRINT 16: 75-78..
20. Irum M (2007) Comparison of phytochemical and chemical control of Fusarium oxysporium f. sp. ciceri. Mycopath 5: 107-110.

21. Abbas HT, Sahi ST, Habib A, Ahmed S (2016) Laboratory evaluation of fungicides and plant extracts against different strains of *Colletotrichum falcatum* the cause of red rot of sugarcane. Pak J Agric Sci 53: 181-186.

22. Shivapuri A, Sharma OP, Jhamaria SL (1997) Fungitoxic properties of plant extracts against pathogenic fungi. J Mycol Plant Pathol 27: 29-31.

23. Ahmed MF, Khalequzzaman KM, Islam MN, Anam MK, Islam MT (2002) Effect of plant extracts against Bipolaris oryzae of rice under *in vitro* conditions. Pak J Biol Sci 5: 442-445.

24. Sharma R, Tamta S (2015) A review on red rot: The "cancer" of sugarcane. Journal of Plant Pathology and Microbiology 5: 1-8.

25. Bernardo-Mazariegos E, Valdez-Salas B, González-Mendoza D, Abdelmoteleb A, Tzintzun Camacho O, et al. (2019) Silver nanoparticles from Justicia spicigera and their antimicrobial potentialities in the biocontrol of foodborne bacteria and phytopathogenic fungi. Rev Argent Microbiol 51: 103-109.

26. Abad LR, D'Urzo MP, Liu D, Narasimhan ML, Reuveni M, et al. (1996) Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization. Plant Sci 118: 11-23.

27. Anumudu CK, Nwachukwu MI, Obasi CC, Nwachukwu IO, Ihenetu FC (2019) Antimicrobial activities of extracts of tobacco leaf (Nicotiana tabacum) and its grounded snuff (utaba) on Candida albicans and Streptococcus pyogenes. J Trop Dis 7: 2.

28. Gwa VI, Nwankiti AO (2017) Efficacy of some plant extracts in *in vitro* control of *Colletotrichum* Species, causal agent of yam (Dioscorea rotundata Poir) tuber rot. Asian J Plant Sci Res 7: 8-16.

29. Radha M, Mala SR, Revathy G, Solayappan AR (1998) *In vitro* antifungal properties of garlic (Allium sativum L.) extracts against *Colletotrichum falcatum* Went. J Biol Control 12: 81-83.