Mycopath (2012) 10(1)**:** 31-36 **Research Article**

Evaluation of methanolic leaf and bark extracts of *Syzygium cumini* against *Ascochyta rabiei*

Muhammad Amin and Arshad Javaid*

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
Corresponding author's email: arshad.iags@pu.edu.pk

Abstract

Laboratory bioassays were carried out to evaluate antifungal activity of different parts of *Sizygium cumin* (L.) Skeels against chickpea (*Cicer arietinum* L.) blight pathogen *Ascochyta rabiei* (Pass.) Lab. Dried and thoroughly crushed leaf, stem-bark and root-bark of the test plant species were extracted in methanol for two weeks. After evaporation of the solvent on a rotary evaporator, different concentrations of methanolic extract viz. 0.5, 1.0, 1.5, …, 4.0% were prepared by dissolving the material first in dimethyl sulphoxide (DMSO) and then in water. Negative control was without any extract or DMSO while positive control contained the same quantity of DMSO as was present in different concentrations of the extracts. In general, all the concentrations of the three types of methanolic extracts significantly reduced ($P \leq 0.05$) fungal biomass to variable extents. The highest antifungal activity was observed due to leaf extract where 23–49% and 20–47% decrease in biomass of *A. rabiei* was recorded over negative and positive control treatments, respectively. Different concentrations of stem-bark extract reduced fungal biomass by 15–23% and 12–20%, and that of root-bark extract reduced fungal biomass by 10–18% and 6–15% over negative and positive control treatments, respectively. This study concludes that methanolic leaf extract of *S. cumini* possess remarkable antifungal potential against *A. rabiei.*

Keywords: *Ascochyta rabiei,* chickpea blight, methanolic extracts, natural fungicides, *Sizygium cumini.*

Introduction

Chickpea is an important cool season leguminous crop cultivated in arid and semi-arid regains of the world (Agarwal *et al.,* 2012). Among legumes, globally it ranks third after *Phaseolus vulgaris* L. and *Pisum sativum* L. (Kanouni *et al.,* 2011), while in Indo-Pak Subcontinent, it ranks first (Sarwar *et al*., 2012). It is an important crop not only because of a major source of protein (Pande *et al.,* 2010), but also has the ability to sustain soil fertility as it adds nitrogen compounds through biological nitrogen fixation by root nodulating rhizobia (Danga *et al.,* 2009). It is cultivated in more than 50 countries with 79% production in Southern and South-Eastern Asia. On global level, it is cultivated on an area of 12 million ha with a production of 10.9 million MT and average yield of 913 kg ha^{-1} in 2010 (FAOSTAT, 2011). In Pakistan, it is grown on an area of 1053.8 thousand ha with total production of 496 thousand tonnes and an average yield of 471 kg ha^{-1} (Anonymous, 2011). Chickpea is susceptible to blight disease caused by *Ascochyta rabiei* that is a highly devastating check for chickpea production around the globe (Chandirasekaran *et al*., 2009; Ali *et al.,* 2012). Generally, the disease affects all parts of shoot of chickpea plants, producing lesions and break them (Bayraktar *et al.,* 2007), causing heavy yield losses. Primary sources of inoculum are infested seeds and debris while air-borne ascospores play an important role in dispersal of the pathogen (Armstrong *et al*., 2001). In Pakistan, 20–25% yield losses in chickpea have been reported under normal conditions in Pakistan. However, in severe form, yield losses may be up to 100% (Pande *et al.,* 2005; Jamil *et al.,* 2010).

Cultivation of resistant chickpea varieties is the most effective mean to control blight disease (Toker and Anci, 2003; Ilyas *et al*., 2007; Iqbal *et al.,* 2010). However, resistance dose not last long because of emergence of new races of the pathogen (Jamil *et al.,* 2010). *A. rabiei* is highly variable and has a number of races (Ilyas *et al*., 2007). Severe Ascochyta blight epidemics have been reported in different chickpea growing regions of the world, including those areas where resistant genotypes have been adopted (Navas-Cortes *et al*., 1998). Therefore, the disease is generally controlled by application of synthetic fungicides. A number of foliar applied fungicides viz. captafol, mancozeb, dithianon, captan, ferbam, Bordeaux mixture, maneb, penconazole, chlorothalonil, propiconazole, tebuconazole, propineb, difenoconazole, azoxystrobin and thiabendazole are known to reduce Ascochyta

blight attack (Nene and Reddy 1987; Shtienberg *et al.,* 2000, 2005; Ahmed *et al.,* 2008). However, synthetic fungicides are not eco-friendly and their indiscriminate use pollutes the environment and causes health hazards (Chiejina and Ukeh, 2012). Because of negative effects of synthetic agro-chemicals on environment and health, scientists are searching alternatives from plants and microbes (Meragelman *et al.,* 2005). Studies have shown that crude plant extracts can control the growth of many fungi such as *Alternaria alternata, Sclerotium rolfsii, Macrophomina phaseolina, Fusarium oxysporum* and others (Riaz et al., 2010; Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Javaid and Samad, 2012). Many potent antifungal compounds have isolated from plants. Jabeen *et al.* (2011) reported that β-amyrin, isolated from leaves of *Melia azedarach* was highly effective against *A. rabiei.* Likewise, Kanwal *et al.* (2011) isolated two flavonoids viz. 7-*O*-glucoside and (–)-epi-catechin from leaves of *Azadirachta indica* which were very effect against *M. phaseolina* and other fungi. Keeping in view the importance of natural plant products as antifungal agents, the present study was designed to evaluate the antifungal potential of methanolic extracts of different parts of *S. cumini* against *A. rabiei.*

Materials and Methods

Leaves, stem-bark and root-bark were collected from a mature *S. cumini* tree growing in University of the Punjab, Lahore, Pakistan. All the three plant materials were thoroughly washed under tap water and dried in sunlight. Two hundred grams of each plant materials were thoroughly crushed and soaked in 1.0 L of methanol at room temperature for 14 days. Materials were passed through muslin cloth and residues were re-extracted with methanol for one week and filtered. Methanol was evaporated on a rotary evaporator and the crude extracts left behind were collected.

For preparation of solutions, 14.4 g methanolic extract of each of the three plant parts were dissolved in 6 mL dimethyl sulphoxide (DMSO). Sterilized distilled water was added to prepare 18 mL of stock solution of each extract. In a similar way, a control solution was prepared by dissolving 6 mL DMSO in 12 mL distilled water. Malt extract broth (76 mL for each treatment) was autoclaved in 250-mL conical flasks. After cooling the flasks at room temperature, different quantities of stock solution (0.5, 1.0, …4.0 mL) and control solution (3.5, 3.0, …0 mL), respectively, were added to each flask to raise the volume to 80 mL in each flask. Positive control treatment received 4 mL of control solution only. Likewise, 4 mL of sterilized distilled water was added in negative control treatment. Medium was divided into four parts in 100-mL flasks each containing 20 mL medium. One actively growing fungal plug of 5 mm diameter was added to each flask. Flasks were incubated in an incubator maintained at 26 °C. Experiment was conducted in a completely randomized design with four replications. Fungal biomass from each flask was filtered on a pre-weighed filter papers after 10 days incubation period, dried at 60 °C and weighed.

All the data were analyzed by analysis of variance followed by LSD Test to compare the treatment means at $P \leq 0.05$ using computer software Statistix 8.1.

Results and Discussion

Analysis of variance (ANOVA) revealed that there was significant effect ($P \leq 0.001$) of plant parts (P) and extract concentrations (C) as well as their interaction (P × C) for biomass of *A. rabiei* (Table 1).

DMSO was used to dissolve the methanolic extracts in the present study. A positive control treatment without extracts was included to assess the effect of DMSO on the fungal growth. In comparison with negative control treatment, there was only 4% reduction in fungal growth due to DMSO in positive control (Fig. 1). Earlier studies have shown variable effects of DMSO on growth of other fungal species namely *A. alternata, S. rolfsii* and *M. phaseolina.* The effect of DMSO on fungal growth generally varies with fungal species and DMSO concentration in the growth medium (Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Javaid and Samad, 2012). Among the three types of extracts, methanolic leaf extract exhibited the maximum antifungal activity against *A. rabiei*. All the concentrations of the extract significantly decreased fungal biomass by 23–49% and 20–47% over negative and positive control treatments, respectively (Fig. 1A & 2). Earlier, Jabeen and Javaid (2010) studied the effect of aqueous, ethanolic and *n*-hexane extracts of *S. cumini* of 1–5% concentrations on radial growth of *A. rabiei* using malt extract agar medium. They reported 7–30% reduction in radial growth of the fungus due to different concentrations of the three types of extracts. Gallic and ellagic acid polyphenol derivative abundantly present in *S. cumini* leaf may possibly be the cause of antifungal activity of the leaf extract against *A. rabiei* (Chattopadhyay *et*

al., 1998; Mahmoud *et al.,* 2001). In addition, kaempferol, myricetin, acylated flavonol glycosides and other polyphenols are present in leaves of *S. cumini* (Mahmoud *et al.,* 2001; Timbola *et al.,* 2002), which could be responsible for antifungal activity (Jabeen and Javaid, 2010).

Methanolic stem-bark extract proved comparatively less effective than leaf extract against *A. rabiei.* Different concentrations of this extract significantly declined fungal biomass by 15–23% and 12–20% over negative and positive control treatments, respectively (Fig. 1B & 2). Root-bark extract proved least effective against the pathogen.

Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Syzygium cumini* on biomass of *Ascochyta rabiei*.

Sources of variation	df	SS	MS	F values
Treatments	29	0.807	0.0278	36*
Plant parts (P)	2	0.257	0.128	165*
Concentration (C)	9	0.448	0.0497	64*
P × C	18	0.103	0.0057	7.3*
Error	90	0.07	0.000779	
Total	120	34.89		

*, Significant at P≤0.001.

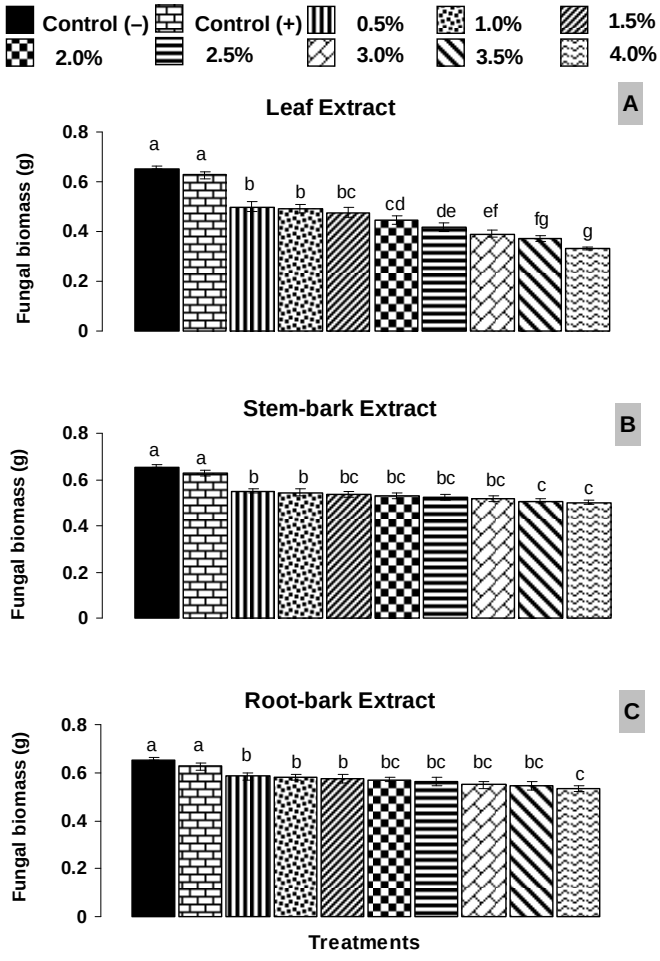

Fig. 1: Effect of different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Syzygium cumini* on growth of *Ascochyta rabiei*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

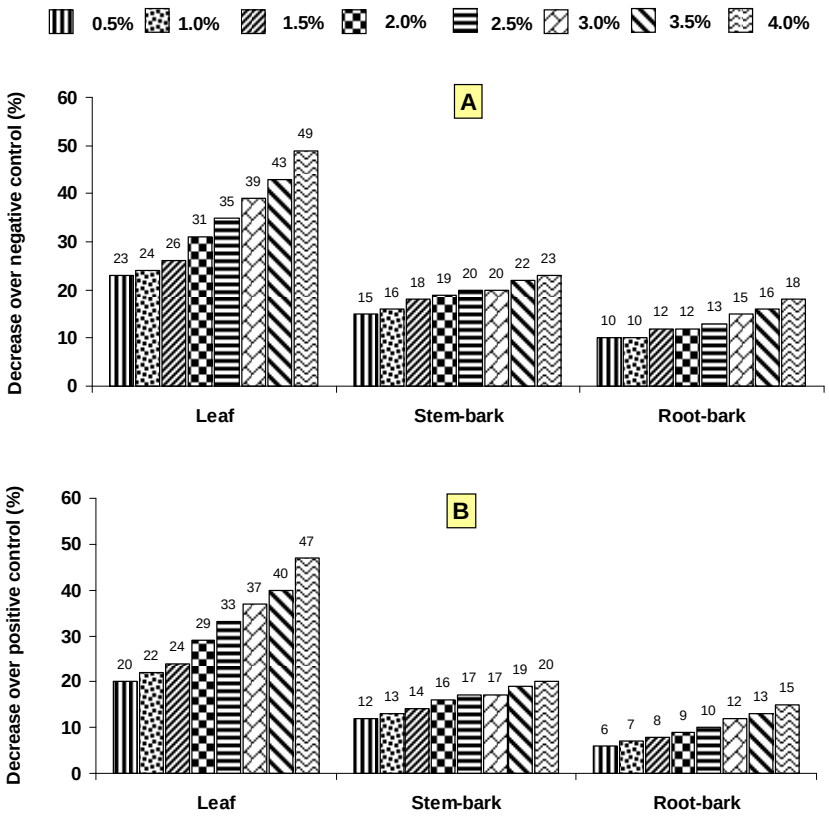

Fig. 2: Percentage decrease in biomass of *Ascochyta rabiei* due to different concentrations of methanolic leaf, stem-bark and root-bark extracts of *Syzygium cumini* over negative and positive control treatments.

There was only 10–18% and 6–15% reduction in fungal biomass due to different concentrations of this extract (Fig. 1C & 2). In a similar study with stem-bark and root-bark aqueous, *n*-hexane and ethanolic extracts, Jabeen and Javaid (2010) reported 21–64% and 23–39% reduction in radial growth of *A. rabiei* on solid malt extract medium. Most of the previous studied on barks of *S. cumini* have been conducted with respect to their medicinal uses (Caceres *et al.,* 1993; Brito *et al.,* 2007), and studies regarding antifungal activity are scars (Jabeen and Javaid, 2010). A number of chemical constituents including resins (gambol), tannins, acids (palmitic, oleic, stearic, gallic), terpans (α-pigeon, limonene, β-pigeon), flavanols, steroids (phytosterol) and saponinic glycosides have been identified from *S. cumini* bark (Albuquerque, 1989). Among these, flavanols, tannins and limonene are known for their antifungal activities (Thirunarayanan, 2003; Sisti *et al.,* 2007; Sharma and Tripathi, 2008). Moreover, triterpenes, especially friedelolactone and friedelin, identified in stem-bark of *S. cumini* exhibited antifungal activity against dermatophytes viz. Trichophyton mentagrophytes, Microsporum audouinii, and Trichophyton soudanense (Kuiate *et al.,* 2006).

Conclusion

It is concluded that methanolic extracts of all the three parts of *S. cumini* possess antifungal potential against *A. rabiei.* However, antifungal activity of leaf extract was the highest. Further studies are needed to isolate the effective antifungal compounds from methanolic leaf extract.

Acknowledgement

Pakistan Science Foundation provided financial support for this study through a research project PSF/NSLP/P-PU (53) entitled "Natural compounds from allelopathic trees as antifungal agents against *Ascochyta rabiei* (Pass.) Lab." under Pak-US Natural Sciences Linkages Programme (NSLP) Endowment Fund.

References

Agarwal G, Jhanwar S, Priya P, Singh VK, Saxena MS, 2012. Comparative analysis of kabuli chickpea transcriptome with desi and wild chickpea provides a rich rResource for development of functional Mmarkers. *PLoS ONE,* 7: e52443.

Ahmed HU, Hwang SF, Gossen BD, 2008. Chemical control of ascochyta blight (*Ascochyta rabiei*) of chcikcpea. *Can. J. Plant Pathol.,* **30**: 367-367.

Albuquerque JM, 1989. Plantas medicinais de uso popular. Brasılia: Associacao Brasileira de Educacao Agrıcola Superior.

Ali H, Alam SS, Attanayake RN, Rahman M, Chen W, 2012. Population structure and mating type distribution of the chickpea blight pathogen *Ascochyta rabiei* from Pakistan and the United States. *J. Plant Pathol.,* **94**: 99-108.

Anonymous, 2011. Agricultural Statistics of Pakistan 2010-11. Statistics Division, Pakistan Bureau of Statistics. Available at: http://www.pbs.gov.pk/content/ agricultural-statistics-pakistan-2010-11. Accessed on 13-09-2013.

Armstrong CL, Chongo G, Gossen BD, Duczek LJ, 2001. Mating type distribution and incidence of the teleomorph of *Ascochyta rabiei* (*Didymella rabiei*) in Canada. *Canadian J. Plant Pathol.,* **23**: 110-113.

Bayraktar H, Dolar FS, Tör M, 2007. Determination of genetic diversity within *Ascochyta rabiei* (Pass.) Labr., the cause of Ascochyta blight of chickpea in Turkey. *J. Plant Pathol.,* **89**: 341-347.

Brito FA, Lima LA, Ramos MFS, Nakamura MJ, Cavalher-Machado SC, Siani AC, 2007. Pharmacological study of anti-allergic activity of *Syzygium cumini* (L.) Skeels. *Braz. J. Med. Biol. Res.,* **40**: 105-115.

Caceres A, Fletes L, Aguilar L, Ramirez O, Figueroa L, Taracena AM, 1993. Plants used in Guatemala for the treatment of gastrointestinal disorders. III: Confirmation of activity against enterobacteria of 16 plants. *J. Ethnopharmacol.,* **38**: 31-38.

Chandirasekaran R, Warkentin TD, Gan Y, Shirtliffe S, Gossen BD, Taran B, 2009. Improved sources of resistance to Ascochyta blight in chickpea. *Can. J. Plant Sci.,* **89**: 107-118.

Chiejina NV, Ukeh JA, 2012. Antimicrobial properties and phytochemical analysis of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* on fungal diseases of tomato fruit. *J. Nat. Sci. Res.* **2**: 10-16.

Danga BO, Ouma JP, Wakindiki IIC, Bar-Tal A, 2009. Legume–wheat rotation effects on residual soil moisture, nitrogen and wheat yield in tropical regions. *Adv. Agron,*, **101**: 315-349

FAOSTAT, 2011. Available online: http://faostat.fao.org/site/567/DesktopDefault.aspx (accessed on January 20, 2012).

Ilyas MB, Chaudhry MA, Javed N, Ghazanfar MU, Khan MA, 2007. Sources of resistance in chickpea germplasm against Ascochyta blight. *Pak. J. Bot.,* **39**: 1843-1847.

Iqbal D, Javaid A, 2012. Bioassays guided fractionation of *Coronopus didymus* for its antifungal activity against *Sclerotium rolfsii. Nat. Prod. Res.,* **26**: 1638-1644.

Iqbal SM, Ali S, Gjafoor A, 2010. Development of resistance in chickpea against *Ascochyta rabiei. Mycopath,* 8: 61-64.

Jabeen K, Javaid A, 2010. Antifungal activity of *Syzygium cumini* against *Ascochyta rabiei,* the cause of chickpea blight*. Nat. Prod. Res.,* **24**: 1158–1167.

Jabeen K, Javaid A, Ahmad E, Athar M, 2011. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei* the cause of chickpea blight. *Nat. Prod. Res.,* **25**: 264-276.

Javaid A, Saddique A, 2012. Control of charcoal rot fungus *Macrophomina phaseolina* by extracts of *Datura metel. Nat. Prod. Res.,* **26**: 1715-1720.

Javaid A, Samad S, 2012. Screening of allelopathic trees for their antifungal potential against *Alternaria alternata* strains isolated from dying back *Eucalyptus* spp. *Nat. Prod. Res.,* **26**: 1697-1702.

Jamil FF, Sarwar M, Sarwar N, Khan JA, Zaid HM, Yousaf S, Imran HM, Haq I, 2010. Genotyping with RAPD markers resolves pathotype diversity in the Ascochyta blight and Fusarium wilt pathogens of chickpea in Pakistan. *Pak. J. Bot.,* **42**: 1369-1378.

Kanouni H, Taleei A, Okhovat M, 2011. Ascochyta blight [*Ascochyta rabiei* (Pass.) Lab.] of chickpea (*Cicer arietinum* L.): breeding strategies for resistance. *Int. J. Plant Breed. Genet.,* **5**: 01-22.

Kanwal Q, Hussain I, Siddiqui HL, Javaid A, 2011. Antimicrobial activity screening of

isolated flavonoids from *Azadirachta indica* leaves. *J. Serb. Chem. Soc.,* **76**: 375-384.

Kuiate JR, Mouokeu S, Wabo HK, Tane P, 2006. Antidermatophytic triterpenoids from *Syzygium jambos* (L.) Alston (Myrtaceae). *Phytother. Res.,* **21**: 149-152

Meragelman TL, Tucker KD, McCloud TG, Cardellina JH, Shoemaker RH, 2005. Antifungal flavonoids from *Hildegardia barteri*. *J. Nat. Prod.,* **68**: 1790-1792.

Navas-Cortes JA, Perez-Artes E, Jimenez-Diaz RM, Llobell A, Brainbridge BW, Heale JB, 1998. Mating type, pathotype, RAPDs analysis in *Didymella rabiei*, the agent of Ascochyta blight of chickpea. *Phytoparasitica*, **26**:199-212.

Nene YL, Reddy MV, 1987. Chickpea diseases and their control. In: The Chickpea'. (Eds. M.V. Saxena and K.B. Singh), CAB International Oxford. pp. 233-270.

Pande S, Siddique KHM, Kishore GK, Gaur PM, Gowda CLL, Bretag TW, Crouch JH, 2005. Ascochyta blight of chickpea (*Cicer arientinum* L.): a review of biology, pathology, and disease management. *Aust. J. Agric. Res.,* **56**: 317–332.

Pande S, Sharma M, Gaur PM, Gowda CLL, 2010. Host Plant Resistance to Ascochyta Blight of Chickpea. Information Bulletin No. 82. Patancheru 502 324 Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. ISBN 978-92-9066-525-0. Order code: IBE 082. pp. 40.

Riaz T, Khan SN, Javaid A, 2010. Management of corm-rot disease of gladiolus by plant extracts. *Nat. Prod. Res.,* **24**: 1131–1138.

Sarwar N, Akhtar KP, Shah TM, Atta BM, 2012. Evaluation of chickpea advance genotypes against blight and wilt diseases under field conditions. *Int. J. Agric. Biol.,* **14**: 993-996.

Sharma N, Tripathi A, 2008. Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiol. Res.,* **163**: 337-344.

Shtienberg D, Vintal H, Brener S, Retig B, 2000. Rational management of *Didymella rabiei* in chickpea by integration of genotype resistance and post infection application of fungicides. *Phytopathology*, **90**: 834-842.

Shtienberg D, Gamliel-Atinsky E, Retig B, Brener S, Dinoor A, 2005. The significance of preventing primary infections by *Didymella rabiei* and development of a model to estimate the maturity of pseudothecia. *Plant Dis.,* **89**: 1027-1034.

Sisti, M., De Santi, M., Fraternale, D., Ninfali, P., Scoccianti, V., & Brandi, G. (2007). Antifungal activity of Rubus ulmifolius Schott standardized in vitro culture. *LWT – Food Sci. Technol.,* **41**: 946-950.

Thirunarayanan G, 2003. Synthesis, characterization and biological activities of someflavone derivatives. *Asian J. Chem.,* **15**: 907-910.

Toker C, Anci H, 2003. Selection of chickpea (*Cicer arietinum* L.) genotypes for rResistance to Ascochyta blight [*Ascochyta rabiei* (Pass.) Labr.], yield and yield criteria. *Turk. J. Agric. For.,* **27**: 277-283.