JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2014. Vol. **8**(5), p. 3937-3943

Antifungal Potential of Propolis Against Carcinogenic Citrinin Produced by *Aspergillus terreus* Thom

Hashem Abeer[1] and Abd_Allah EF[2]*

[1]Department of Botany and Microbiology, Faculty of Science,
King Saud University, Riyadh 11451, Saudi Arabia.
[2]Department of Plant Production, Faculty of Food and Agricultural Sciences,
King Saud University, Riyadh - 11451, Saudi Arabia.

(Received: 10 August 2014; accepted: 21 September 2014)

A laboratory experiment was conducted to examine the propolis-induced changes on *Aspergillus terreus* as common producer of carcinogenic citrinin in seeds, feed, food and foodstuff. Keeping in view the attributes of growth criteria and citrinin production of *A. terreus*, propolis was very effective significantly in decreasing mold growth and mycotoxin production. The alterations in elements accumulation were negatively due to impact of propolis. The amino acids metabolism as key physio-biochemical attributes of fungal metabolism directly influenced by propolis negatively. The accumulation of both glutamic acid and proline due to propolis was observed, signalize to the activation of glutamate kinase followed by glutamine synthetase (for glutamic acid) and ^{3}-semialdehyde dehydrogenase (for proline), respectively. The alteration in amino acids metabolism indicated clearly too significant modifications in mold metabolic process towards catabolism.

Key words: Propolis; Aspergillus terreus; Citrinin; amino acids; elements accumulation.

Aspergillus terreus is the major producer of carcinogenic citrinin in crops worldwide in addition to its importance as opportunistic human pathogen in aspergillosis (Pasqualotto, 2009; Horn and Moore, 2009; Hashem *et al*., 2013). The common clinical syndromes caused by *A. terreus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation of the fungus (Hedayati *et al*., 2007). However, balanoposthitis caused by *A. terreus* has rarely been reported. In this report, we describe a case of asevere penile *A. terreus* infection, as well as the treatment administered to this patient (Li *et al*., 2005). Antifungal resistance is a broad concept describing failure of a fungal infection to respond to antifungal therapy (Kontoyiannis and Lewis, 2002). Consequently, there is urgent need to develop nonchemical alternative strategies to dissolve this clinical problem via alternative means with biotic origin.

Propolis [PR] (Bee glue) is a mixture of bee wax and resins collected by the honeybee (*Apis mellifera*) from parts of plant, buds, flower and exudates (Ghisalberti, 1979). Propolis has been used to seal holes, exclude draught, and protect the beehive against external invaders. The main function of propolis is to prevent the decomposition of organic matter within beehive by inhibiting microbial growth and activity (Quiroga *et al*., 2006). Many biological activities

* To whom all correspondence should be addressed.
E-mail: eabdallah@ksu.edu.sa

of propolis have been reported such as medicinal (Orsolic *et al*., 2003) antibacterial (Uzel *et al*., 2005), antifungal (Buchta *et al*., 2011; Hashem *et al*., 2013) and antitumor (Frozza *et al*., 2013). Generally, many biological active compounds have been identified in propolis such as polyphenols, phenolic aldehyde, sequiterpene quinines, coumarins, amino acids, steroids, and inorganic compounds (Katircioglu and Mercan, 2006). The biological activities of propolis vary depend up on geographical origin and bearing plants (Kujumgiev *et al*., 1999).

The objective of this study was to observe the antifungal impact of effect of propolis on growth and some metabolic process of *A. terreus* under laboratory conditions.

MATERIALS AND METHODS

The experimental microorganisms

Aspergillus terreus Thom was isolated from rice grain sample collected from Zagazig city, Egypt. The identification of experimental mold was carried out according to Domsch *et al*., (1993). Citrinin-sensitive strain of *Bacillus brevis* was kindly provided by Dr. Gamal El-Didamony (Botany Department, Faculty of Science, Zagazig University, Egypt) was used as biological indicator of citrinin.

Preparation of PR extract

Propolis was collected from colonies at Abha city, Saudi Arabia and it was scrappedoff the top of frames and inner wall boxes of bee colonies. PR was extracted with aqueous:ethanol (10:90, v/v) as described by Hashem *et al*., (2012). Based on preliminary experiment, three concentrations (0.0, 0.3 and 0.5; g/100ml) of PR were selected.

Culture growth media and general growth conditions of *A. terreus*

Czapek-Dox agar medium (Raper and Fennel, 1965) was used for the growth of experimental mold (*A. terreus*). Broth cultures (100 ml medium in 250 ml capacity Erlenmeyer flasks) were inoculated with 0.5 mm agar disc of *A. terreus* as described by Abd_Allah and Ezzat (2005). Mycelial dry weight was estimated by filtration the broth mold culture after ten days of incubation at 28+ ^{o}C in dark at static state. Mycelium growth was washed carefully with distilled water, dried at 105oC up to two successive constant weights, then the mycelia dry weight was recorded. The test fungus was also grown in agar medium in 9.0 cm diameter Petri-dishes for seven days incubated at 28+ ^{o}C in dark and the radial growth was measured. Conidial production and their germination were studied according to Roberts and Selitrenikoff (1988).

Estimation of mycotoxin citrinin

The extraction and clean-up of citrinin were carried out according to Jackson and Ciegler (1978). Fifty ml of culture filtrate was shaken with an equal volume of chloroform for 30 min. The chloroform layer was separated over a bed of anhydrous sodium sulfate. Clean-up was carried out using concentrated HC1 and 0.1 M $NaHCO_3$. The quantitative determination of citrinin was carded out fluorometrically according to Trantham and Wilson24 using spectrofluorometer Varian model 330 with detector (Varian Associates, ouston, TX, USA). The excitation wavelength of the fluorometer was 330 nm and the emission wavelength was 500 nm. The chemical and biological confirmations were carries out according to Hald and Krogh (1973) and Abd_Allah and Ezzat (2005), respectively.

Estimation of ion accumulation

The oven dry mycelial samples of *A. terreus* were digested and the ions were estimated according to the method of Wolf (1982) using a flame photometer Jenway Flame Photometer, Bibby Scientific Ltd-Stone-Staffs-St15 0SA – UK. Standard curve of each mineral (10- 100 µg/ml) used as reference.

Amino acids analysis

Free amino acids were extracted from dry mycelial samples of *A. terreus* with absolute ethanol. The qualitative as well as quantitative determination of amino acids was carried out using LKB 415 alpha plus Amino Acid Analyzer (AAA) according to Christias *et al*., (1975). Standard amino acids (BHD Chemicals, Poole, UK) were used as reference.

Statistical analysis

All data were subjected to statistical analysis. Treatments were compared using Fisher's Least Significant Difference (LSD) analysis. Cluster analysis was performed using SPSS 20 software package.

RESULTS

In the current study, a preliminary test was carried out to elucidate the antifungal potential of PR on local (Egyptian) isolate of *A. terreus* isolated from rice grain sample. Such isolate was able to produce citrinin *in vitro* and *in vivo* (in rice grains). It is necessary to mention that such rice grain sample was contaminated with mycotoxin citrinin.

Data recorded for different criteria related attributes growth of *A. terreus* showed that application of varying levels of PR (0.0, 0.3, and 0.5 g/100ml) caused significant decrease in growth criteria was directly proportional with the concentration of PR (Table1). The concentration of 0.3 (g/100 ml) of PR caused significant decrease in conidial production; conidial germination; radial growth and mycelial growth of *A. terreus* by percent of 22.25; 39.29; 17.44 and 37.74, respectively (Table 1). The application of further concentration of PR (0.5, g/100 ml) was accompanied with significant inhibition in all growth criteria compared with both the previous concentration (0.3, g/100 ml) and control (Non) as shown in Table 1.

PR stress (0.0, 0.3, and 0.5 g/100ml) significantly reduced the production of mycotoxin citrinin in solid media [Petri dishes] and liquid media [conical flasks] (Figure 1). Citrinin production by *A. terreus* in both Petri dishes and conical flasks was inhabited significantly under concentration of (0.3, g/100 ml) of PR. At the further concentration of PR (0.5, g/100 ml), significant inhibition in production of citrinin in conical flasks was observed, whilst citrinin production in Petri dishes completely inhibited (Fig. 1).

The results for element contents in the mycelial growth *A. terreus* under the biotic stress of PR were shown in Table 2. Mycelial Na^+contents increased considerably (p50.01) in *A. terreus* under all PR levels and a maximum increase in this ion was observed at 0.5 g/100ml (Table 2). A considerable reduction in K^+concentrations was observed under PR stress, a maximum reduction in $K^+$was observed at 0.5 g/100ml. Mycelial Ca^{2+}contents were also affected due to imposition of varying levels PR stress to the growth medium. In the same context, the Mg^{2+} contents were significantly decreased in directly proportional with PR concentration (Table 2).

The chromatographic analysis revealed the presence of 20 free amino acids in mycelial control of *A. terreus*. The amino acids were alanine, arginine, asparagine, aspartic acid, cysteine,

Table 1. Effect of different concentrations of propolis (w/v) on number of germinated conidia (conidia in number); conidial production (conidia mm^2X10^4); radial growth (cm/plate) and mycelial growth (g/100 ml culture medium) of *A. terreus*

Concentration of propolis (g/100 ml)	Growth criteria			
	Number of germinated conidia (out 100 conidia)	Conidial production (conidia m^2X10^4)	Radial growth (cm/plate)	Mycelial growth (g/100 ml)
Control (Non)	95.34	9.34	9.00	2.04
0.3	74.12	5.67	7.43	1.27
0.5	35.87	1.25	3.72	0.64
LSD at: 0.05	10.14	0.86	1.23	0.38

Table 2. Effect of different concentrations of propolis (w/v) on elements accumulation (mg/g dry wt) of *A. terreus*.

Propolis (g/100 ml)	Elements accumulation (mg/g dry wt)			
	Sodium	Potassium	Calcium	Magnesium
Control (Non)	0.182	2.573	0.132	0.307
0.3	0.237	1.782	0.077	0.183
0.5	0.416	1.043	0.034	0.104
LSD at: 0.05	0.07	0.23	0.04	0.08

Table 3. Effect of different concentrations of propolis (w/v) on amino acids compositions (mg/g dry wt) of *A. terreus*.

Propolis(g/100 ml)	Free amino acid profile of *A. terreus* (mg/g)																		
	Alanine	Arginine	Asparagine	Aspartic acid	Cysteine	Glutamic acid	Glutamine	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine	Total
Control (Non)	0.41	0.58	1.02	0.16	0.08	0.15	0.82	0.12	0.11	0.18	0.34	0.64	0.07	0.43	0.24	0.48	0.13	0.17	0.63	0.21	18.46
0.3	0.21	0.93	1.95	0.97	0.04	2.33	1.64	1.16	0.81	0.47	1.47	0.42	0.0	1.62	0.67	1.27	1.05	0.42	1.25	1.09	33.97
0.5	0.58	1.66	2.36	1.30	0.0	4.75	2.38	2.01	1.33	0.72	2.81	0.24	0.0	2.09	1.83	2.34	2.15	0.59	2.07	1.98	52.07
LSD at 0.05	0.12	0.21	0.24	0.48	0.02	0.73	0.34	0.43	0.41	0.17	0.62	0.23	0.01	0.52	0.31	0.58	0.27	0.11	0.43	0.71	

glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, Tyrosine and valine in concentration of 0.41, 0.58, 1.02, 0.16, 0.08, 0.15, 0.82, 0.12, 0.11, 0.18, 0.34, 0.64, 0.07, 0.43, 0.24, 0.48, 0.13, 0.17, 0.63 and 0.21, respectively with total free amino acids equal 18.46 mg/ g mycelial dry weight (Table 3). As shown in Table 1, the application of PR at concentration of 0.3 g/100ml caused the absence of methionine, and significant decrease in alanine, cysteine and lysine by percent of 48.7, 50.0 and 34.3 respectively as compared with control *A. terreus*. On the other hand, the same concentration of PR (0.3 g/ 100ml) caused significant increase in total free amino acids of *A. terreus* was accompanied with significant increase in the rest amino acids. The higher concentration of PR (0.5 g/100ml) caused significant increase in total free amino acids by percent of 108.0, as compared with control *A. terreus*. Such increase in total free amino acids caused by PR at concentration of (0.5 g/ 100ml) was accompanied with significant increment in all free amino acids except cysteine as well as methionine which were disappeared (not detected under the experimental conditions) and lysine which decreased by percent of 62.5 as compared with control *A. terreus*.

DISCUSSION

PR (or bee glue) is a natural resinous substance produced by *Apis mellifera* bees made up from parts of the plants, buds and exudates. In the beehive, PR is thought to be used to seal holes,

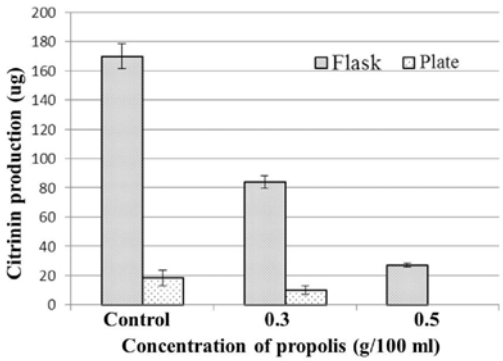

Fig. 1. Effect of different concentrations of propolis (w/v) on citrinin production [(µg/100 culture media) for flask and (µg/Plate) for plate] of *A. terreus*.

exclude draught and protect against external invaders. Its main function, however, is to prevent the decomposition of organic matter within the hive by inhibiting microbial growth (Quiroga *et al*., 2006). Long ago, it was reported that PR was very well known to the priests who had monopolized medicine, chemistry and art of mummifying corpses (Lotfy, 2006). In the present study, the criteria related attributes growth of *A. terreus* inhabited significantly due to application of PR. Similar antifungal activities of PR on growth of different fungi such as *Trichophyton mentagrophytes* (Buchta *et al*., 2011); *Candida albicans* (Afrouzan *et al*., 2012); *Aspergillus parasiticus* (Hashem *et al*., 2012) and *Penicillium viridicatum* (Hashem *et al*., 2013) have been reported. The phenols (Ghaly *et al*., 1998) and flavonoids (Cushnie and Lamb, 2005) has been considered the main carriers of antifungal properties of PR. Also, it has been reported that PR might inhibit DNA replication hence caused an inhibition in cell division Takaisi-Kikuni and Schilcher (1994). As another suggested mechanism, clear catabolic repression of lipids metabolism (total lipids, neutral lipids, phospholipids) of *A. parasiticus* has been reported by PR in our previous study (Hashem *et al*., 2012). Moreover, Hashem *et al*., (2013) reported that PR caused significant alteration in amino acids metabolism of *Penicillium viridicatum* towards clear catabolic repression. A marked reduction in production of citrinin in both solid media and liquid media by *A. terreus* was observed due to different PR concentrations (0.0, 0.3 and 0.5 g/100ml). Although mycotoxins reduction production by most mycotoxigenic fungi is a common effect of PR stress (Khezri *et al*., 2006; Li *et al*., 2007; Hashem *et al*., 2012; Hashem *et al*., 2013), however, the actual physiological and biochemical mechanisms involved in mycotoxins reduction are still not well especially according to molecular approach. The accumulation of Na+ contents in mycelia of *A. terreus* increased considerably under PR stress, however K^+, Ca^{2+} and Mg^{2+} contents were significantly decreased in directly proportional with PR concentrations. The accumulation excessive accumulation of Na+ ions in fungal mycelia under biotic and abiotic stress is defense mechanism (Munns and Tester, 2008; Alqarawi *et al*., 2012; Souza *et al*., 2013) which further leads to alteration of mycelia osmotic potential thereby reducing cell turgidity in fungal cells due to water loss caused by PR (Sripriya *et al*., 2009). Study done by Mello *et al*., (2006) suggested that the antifungal activity of propolis is due to changes in the cell wall permeability leading to disturbance of volume and hence membrane rupture. In the same context, it was reported that Release of cytochrome c from mitochondria is therefore considered a key initial step in the apoptotic process (Ott *et al*. 2002). Furthermore, de Castro *et al*., (2011) reported that propolis was able to induce an apoptosis cell death response of *Saccharomyces cerevisiae* via alteration of cytochrome *c*. The potential of PR to increase the direct potential-dependent permeabilization of biological membranes suggest it to use as anticancer polyarginine-KLA peptides (Lemeshko, 2013). The data indicated the presence of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine amino acids in mycelial control of *A. terreus*. This result confirmed those of Vyas *et al*., (2005); Alqarawi *et al*., (2012); Hashem *et al*., (2012) who reported parallel results. PR stress remarkably enhanced the absence of methionine, and significant decrease in alanine, cysteine and lysine, however glutamate amino acids (glutamic acid and proline) and total free amino acids of *A. terreus* significantly increased. The consumption of such amino acids decreased due to PR to support more energy (Adenosine Tri-phosphate, ATP) required for mold resistance against antifungal potential as described by Yamaguchi and Fujimura (2005); Chen *et al*., (2008) and Alqarawi *et al*. (2012). The accumulation of glutamic acid and proline due to PR means activation of their biosynthesis from glutamate via both glutamate kinase followed by ^{3}-semialdehyde dehydrogenase (for proline) and glutamine synthetase (for glutamic acid), respectively as reported by Albert *et al*. (2002). Such alteration in amino acids metabolism is parallel to Perea and Patterson (2002); El-Mehalawy *et al*., (2008); AbdEl-Ghany *et al*., (2009) and Hashem *et al*., (2012) who reported that such alteration in amino acids metabolism as sensitive monitor for antifungal resistance against abiotic stress of fungicides. In another study on *Aspergillus flavus*, Alqarawi *et al*., (2012) found that the antifungal from biological

origin caused significant increase in glutamic acid, proline, serine, leucine, phenyl alanine and total free amino acids. Hashem *et al*., (2013) reported similar evidences regard the effect of PR on the amino acids composition of *Penicillium viridicatum*.

In conclusion, PR caused a marked suppression in growth criteria, production of mycotoxin citrinin and induced significant alterations in minerals accumulation and amino acids metabolism of *A. terreus* towards catabolism. It is now evident that PR can be used as alternative biotic antifungal with good and acceptable results will be suitable for future prospects.

ACKNOWLEDGEMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-271.

REFERENCES

1. Abd_Allah EF and Ezzat SM. Natural occurrence of citrinin in rice grains and its biocontrol by *Trichoderma hamatum*. *Phytoparasitica*, 2005; **33**: 73- 84.
2. AbdEl-Ghany TM, AbdEl-Mongy M and Afify MM. Dynamic changes in amino acids and volatile metabolites of soil fungus *Aspergillus flavus* as a result of chloropyrifose methyl application. *J. Appl. Sci. Res*. 2009; **5**: 1-8.
3. Afrouzan H, Amirinia C, Mirhadi SA, Ebadollahi A, Vaseji N and Tahmasbi G. Evaluation of antimicrobial activity of propolis and nanopropolis against *Staphylococcus aureus* and *Candida albicans*. *Afr. J. Microbiol. Res.*2012; **6**: 421-425.
4. Albert GM, John WF and Michael PS. Biosynthesis and metabolism of amino acids. In: Microbial Physiology 4th Edition, by Wiley-Liss, Inc. 2002; ISBN: 0-471-39483-1, pp. 736.
5. Alqarawi AA, Abd_Allah EF and Hashem Abeer. Effect of *Ephedra alata* on nucleic acids and nitrogen metabolism in *Aspergillus flavus*. *Pak. J. Bot*. 2012; **44**(1): 425-428.
6. Buchta V, erný J and Opletalová V. *In vitro* antifungal activity of propolis samples of Czech and Slovak origin. *Cent. Eur. J. Biol*. 2011; **6**: 160-166.
7. Chen Y, Hengkui L, Changjun C and Mingguo Z. Sensitivity of *Fusarium graminearum* to fungicide JS399-19: *In vitro* determination of baseline sensitivity and the risk of developing fungicide resistance. *Phytoparasitica*, **36**: 326-337.
8. Christias C, Couvaraki C, Georgopoulos SG, Macris B and Vomvoyanni V . Protein content and amino acid composition of certain fungi evaluated for microbial protein production. *Appl. Microbiol*. 1975; **29**: 250-254.
9. Cushnie TPT and Lamb AJ. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Ag*. 2005; **26**: 343-356.
10. De Castro IP, Martins LM and Loh SH. Mitochondrial quality control and Parkinson's disease: a pathway unfolds. *Mol. Neurobiol*. 2011; **43**: 80-86.
11. Domsch KH, Gams W and Anderson TH (1993). Compendium of Soil Fungi. Academic Press, London, pp. 860.
12. El-Mehalawy AA, Gebreel HM, Rifaat HM, El-Kholy IM and Humid AA. Effect of antifungal compounds produced by certain bacteria on physiological activities of human and Plant-pathogenic fungi. *J. Appl. Sci. Res*. 2008; **4**: 425-432.
13. Frozza CO, Garcia CS, Gambato G, De Souza MD, Salvador M, Moura S, Padilha FF, Seixas FK, Collares T, Borsuk S, Dellagostin OA, Henriques JA, Roesch-Ely M. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. *Food Chem. Toxicol*.2013; **52**: 137-42.
14. Ghaly MF, Ezzat SM and Sarhan MM. Use of propolis and ultra- griseofulvin to inhibit aflatoxigenic fungi. *Folia Microbiol*. 1998; **43**:156-160.
15. Ghisalberti EL. Propolis: A review. *Bee World*, 1979; **60**: 59-84.
16. Hald B and Krogh P . Analysis and chemical conrmation of citrinin in barley. *J. Assoc. Off. Anal. Chem*. 1973; **56**: 1440-1443.
17. Hashem Abeer, Abd_Allah EF and Alwathnani Hend A . Effect of propolis on growth, aflatoxins production and lipid metabolism in *Aspergillus parasiticus* Spear. *Pak. J. Bot*. 2012; **44**(3): 1153-1158.
18. Hashem Abeer, Abd_Allah EF and Al Othman Monera . Growth and production of citrinin by *Penicillium viridicatum* Westling and their control by propolis. *J. Pure & Appl. Microbiol*. 2013; **7**(3): 1917-1924.
19. Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P, Denning DW . *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. *Microbiol*. 2007; **153**: 1677-1692.

20. Horn BW and Moore GG . Sexual reproduction in *Aspergillus flavus*. *Mycologia,* 2009; **101**(3): 423-429.
21. Jackson LK and Ciegler A. Production and analysis of citrinin in corn. *Appl. Environ. Microbiol*. 1978; **36**: 408-411.
22. Katircioglu H and Mercan N . Antimicrobial activity and chemical compositions of Turkish propolis from different region. *Afr. J. Biotechnol*. 2006; **5**: 1151-1153.
23. Khezri M, Rostami S, Riseh RS and Alizadeh A . Effect of propolis and clotrimazole on controlling aflatoxin in pistachio (*Pistacia vera* L.). *Inter. J. Agri. Biol*. 2006; **5**: 606-608.
24. Kontoyiannis DP and Lewis RE. Antifungal drug resistance of pathogenic fungi. *Lancet*, 2002; **359**: 1135-1144.
25. Kujumgiev A, Tsvetkova I, Serkedjieva Yu, Bankova V, Christov R and Popov S . Antibacterial, antifungal and antiviral activity of propolis from different geographic origin. *J. Ethnopharmacology,* 1999; **64**: 235-240.
26. Li G, Li B, Yang T, Yin J, Qi H, Liu G. and Zhang G. Sesterterpenoids, terretonins A-D, and an alkaloid, asterrelenin, from *Aspergillus terreus*. *J. Natural Products*, 2005; **68**: 1243-1246.
27. Li X, Schuler MA and Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Ann. Rev. Entomol*. 2007; **52**: 231-253.
28. Lotfy M. Review: Biological activity of bee propolis in health and disease. *Asian Pacific J. Cancer Prevention*, 2006; **7**: 22-31.
29. Lemeshko VV . Electrical potentiation of the membrane permeabilization by new peptides with anticancer properties. *Biochim. et Biophys. Acta*, 2013; **1828**: 1047-1056.
30. Mello AM, Gomes RT, Lara SR, Silva LG, Alves JB, Cortes EM, Abreu SL, Santos VR. The effect of Brazilian Propolis on the germ tube formation and cell wall of *Candida albicans*. *Pharmacologyonline,* 2006; **3**: 352-358.
31. Munns R and Tester M. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol*. 2008; **59**: 651-681.
32. Munns R and Tester M. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol*. 2008; **59**: 651-681.
33. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B and Orrenius S. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. USA*. 2002; **99**:1259–1263.
34. Quiroga EN, Sampietro DA, Soberon JR, Sgariglia MA and Vattuone MA. Propolis from the northwest of Argentina as a source of antifungal principles. *J. Appl. Microbiol*. 2006; **101**: 103-110.
35. Pasqualotto AC. Differences in pathogenicity and clinical syndromes due to *Aspergillus fumigatus* and *Aspergillus flavus*. *Medical Mycol*. 2009; **47**: 261-270.
36. Perea S and Patterson TF. Antifungal resistance in pathogenic fungi. *Clin. Infec. Dis*. 2002; **35**: 1073-1080.
37. Raper KB and Fennell DI . The Genus Aspergillus, pp 686 Baltimore: 1965; Williams & Wilkins Company.
38. Roberts WK and Selitrenikoff CP. Plant cell and bacterial chitinase differ in antifungal activity. *J. Gen. Microbiol*. 1988; **134**: 169-176.
39. Souza ICL, do Nascimento MF, Neta RG de S, Dos Santos JC, Costa LP, Cardoso JC and de Albuquerque-Júnior RLC. Effect of the maltodextrin-induced chemical reticulation on the physical properties and healing potential of collagen-based membranes containing Brazilian red propolis extract. *Inter. J. Medicine Medical Sci*. 2013; **5**(12): 514-524.
40. Sripriya P, Vedantam LV and Podile AR. Involvement of mito-Chondria and metacaspase elevation in harpin Pss-induced cell death of *Saccharomyces cerevisiae*. *J. Cell. Biochem*. 2009; **107**: 1150–1159.
41. Takaisi-Kikuni NB and Schilcher H. Electron microscopy and microcalorimetric investigations of the possible mechanism of the antibacterial action of a defined propolis provenance. *Planta Medica*., 1994; **60**: 222-227.
42. Uzel A, Sorkun K, Önçag Ö, ÇogÔlu D, Gençay Ö and Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. *Microbiol. Res*. 2005; **160**: 189-195.
43. Vyas A, Vyas D and Vyas KM. Production and optimization of cellulases on peretreated groundnut shell by *Aspergillus terreus* AV49. *J.S.I.R.* 2005; **64**: 281-286.
44. Wolf B . A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Anal*. 1982; **13**: 1035-1059.
45. Yamaguchi I and Fujimura M. Recent topics on action mechanisms of fungicides. *J. Pestic. Sci*. 2005; **30**: 67-74.