Antifungal activity of recombinant thanatin in comparison with two plant extracts and a chemical mixture to control fungal plant pathogens

Mojtaba Mamarabadi1*, Abbas Tanhaeian2 and Younes Ramezany1

Abstract

The most common method for controlling plant diseases is the application of chemical pesticides and sometimes use of resistant cultivars. Due to the effects of chemical pesticides on human and environmental health, mutation in pathogens and resistance to various toxins besides the challenges with resistant cultivar production, the constant use of these methods are not recommended any longer. Thus, use of biological control agents along with the natural ingredient extracted from plants and application of peptide with antimicrobial activity, have been the focus of many researchers. In the present study, the antifungal activity of two plant extracts named Turmeric and Persian lilac in comparison with a chemical mixture and recombinant thanatin were evaluated against five following fungal plant pathogens; Geotrichum candidum, Botrytis cinerea, Rhizoctonia solani, Alternaria tenuissima and Gibberella fujikuroi. The results showed that, all treatments have antifungal activity against tested fungi. Both plant extracts were shown an acceptable antifungal activity against tested fungi but their inhibition effects was not comparable with chemical mixture. Turmeric showed a higher rate of mycelial inhibition than Persian lilac. Amongst all treatment, thanatin showed a great antifungal activity by its application at µg level under both in vitro and in vivo condition. Considering to the compatibility of thanatin with human health and environmental safety we could imagine a clear perspective for the application of this recombinant peptide in sustainable agriculture.

Keywords: Phytopathogenic fungi, Plant diseases control, Antimicrobial peptides, Tomato early blight

Introduction

The main source of income for the half people from the world’s population comes from agriculture. The damage caused by plant pathogen and pest makes a significant failure in the grower’s income.

The pesticides are used to prevent the reduction of crop production each year. Evaluating the environmental risks associated with pesticide application indicates that these problems have been increasing over time and this is because of the increasing amount of research on the environmental impacts of pesticides in the world (Pingali and Roger 2012). The most common method for controlling plant pests and diseases in sustainable agriculture is the minimal application of chemical pesticides and using resistant cultivars as well (Stenberg 2017). Due to the effects of chemical pesticides on human and environmental health, mutation in pathogens and resistance to various toxins, the continued use of chemical and synthetic pesticides is not a suitable option (Carvalho 2017). Moreover, there are numerous challenges in resistant cultivar production (kossmann 2012). Thus, other solutions such as using biological control agents along with further consideration on the plant natural ingredients were suggested. Worldwide studies have shown that plant extracts have a high ability to inhibit the growth of microorganisms, and in this regard, medicinal plants have been widely used as antimicrobial agents (Hafidh et al. 2011).
Turmeric or “Indian saffron” (*Curcuma longa* L.) is a plant native to Southeast Asia and belongs to the family Zingiberaceae (Aggarwal et al. 2007; Avanco et al. 2017). The rhizomes of this plant is rich in curcumin which is a natural pigment with important biological activity (Martinez-Correa et al. 2017). Turmeric has been observed to be toxic against fungal pathogens caused different plant diseases (Ferreira et al. 2013; Avanco et al. 2017). We also used Persian lilac as another plant extract with antimicrobial activity. Persian lilac (*Melia azedarach* L.) is one of the most useful plants used in various parts of traditional medicine and belongs to the family of Meliaceae (Saleem et al. 2008; Neycee et al. 2012). The reason for the use of Persian lilac extract in the present study was the likelihood and potential of these compounds for plant pathogen inhibition. For example, the anti-growth effects of this plant extract have been confirmed and reported on *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer* (Sen and Batra 2012).

Besides the antimicrobial effect of plant extracts for fungal inhibition, other alternative methods like use of antimicrobial peptides (AMPs) have also been considered. Many attention have been paid by the researchers on AMPs in recent years. This is due to their efficiency against different pathogens. These peptides are found in nature and have been isolated from a wide range of organisms. Many plants and animals have been manipulated with AMPs encoding genes and several pesticides have been produced based on these peptides. There are many successful examples for application of these peptides in agriculture which are indicated a promising future for extensive application of AMPs (Zasloff 2002; Montesinos 2007; Keymanesh et al. 2009).

Among different Amps, a recombinant thanatin has been synthetized and evaluated in this study. Thanatin was originated from the spined soldier bug (*Podisus maculiventris*). It is the first inducible insect peptide with a broad range of activity against bacteria and fungi at physiological concentrations. Thanatin contains two amino acids including two cysteine residues that form a disulfide bridge (Mandard et al. 1998). So far, several studies have been performed on this peptide. For example, the transgenic rice encoded thanatin was resistant to blast disease in field evaluations (Imamura et al. 2010). Another study showed that the transgenic *Arabidopsis* encoded thanatin was resistant to the fungal and bacterial pathogens (Wu et al. 2013).

Development of transgenic plants with the ability of AMPs production has been proposed as a method to protect plants against pathogens. Despite the benefits of transgenic plants, these plants have some limitations and disadvantages that should not be ignored (Wolfenbarger and Phifer 2000; Andow and Zwahlen 2006). Therefore, due to the lack of permanent access to Amps sources and the associated challenges with transgenic plants, other production platforms resulting recombinant peptide were suggested. One of the most famous platforms for production of recombinant peptides is human embryonic kidney cells (HEK293) which are widely used as a powerful tool for expressing recombinant proteins (Thomas and Smart 2005; Hu et al. 2018).

In this study, HEK293 transfected in our previous study (Tanhaeian et al. 2018) was used for the synthesis and release of recombinant thanatin into medium. In order to demonstrate the effectiveness of recombinant thanatin against fungal plant pathogens, it is necessary to compare the efficacy of this recombinant peptide with other materials having antifungal activity. For this reason, we have compared the efficacy of two plant extracts and a chemical mixture along with recombinant thanatin against five fungal plant pathogens. The results indicated that, all treatments have shown antifungal activity against tested fungi. Amongst all treatments, thanatin showed a good antifungal activity even by its application at µg level under in vitro and in vivo condition.

**Materials and methods**

**Preparation of recombinant thanatin**

Thanatin gene cloning processes was performed according to the same protocol which we used in our recent publication (Tanhaeian et al. 2018). Briefly, the plasmid vector harboring synthetic thanatin encoding sequence (shown in a Additional file 1) and pcDNA™3.1(+) vector (Thermo Fisher Scientific, USA) were digested using *Bam*HI restriction enzyme (Thermo Fisher Scientific, USA). Digested products were purified by gel extraction kit (Thermo Fisher Scientific, USA), and treated by *Xba*I restriction enzyme (Thermo Fisher Scientific, USA) for 5 h; retrieved from the gel and used for ligation by fast ligation kit (Thermo Fisher Scientific, USA). 10 µl of ligation product was then transformed into DH5α competent cells (Invitrogen, USA). The transformed colonies containing the recombinant plasmid (pcDNA™3.1(+) - thanatin) were cultured and subjected for plasmid extraction using a plasmid extraction kit (Thermo Fisher Scientific, USA). Transformation process was confirmed by restriction mapping and sequencing.

The adherent Human embryonic kidney 293 (HEK293) cell line was kindly provided by Department of Animal science, Ferdowsi University of Mashhad, maintained at 37 °C, 5% CO₂ and 5% humidity in Dulbecco’s modified Eagle’s medium (DMEM/F12, Sigma Aldrich, USA) supplemented with 10% fetal bovine serum (FBS) (Fetal FCS; serum from Gibco, USA) and 1% of the penicillin–streptomycin (Invitrogen, USA) as antibiotics. For transfection, the cells were cultured in 35 mm petri dishes.
and allowed to grow until making 50–80% confluent culture. Calcium phosphate co-precipitation procedure was performed in DMEM/F12 medium supplemented with 10% FBS and 1% of penicillin–streptomycin. Recombinant vector and pcDNA™3.1(+) (without insertion, as a negative control) were transfected in separate dishes and then were selected using the medium containing 400 mg/mL Genenticin (Sigma-Aldrich, Germany) for 2 weeks. After antibiotic therapy, the transfected cells containing secretive thanatin were collected from the cultivated medium and stored at 5 °C.

**SDS-PAGE analysis**

30 µL from the supernatant upon transfected cells were run on SDS-PAGE in Tris/glycine/SDS buffer using 15% polyacrylamide gels alongside to a low molecular protein ladder, industrial thanatin and negative control sample (Fig. 1). Thanatin was separated on SDS-PAGE and visualized by silver nitrate according to the staining protocol suggested by manufacturer. The quantification of peptide band was carried out by NIH Image software (ImageJ 1.34s; [http://rsb.info.nih.gov/ij](http://rsb.info.nih.gov/ij)).

**Preparation of aqueous extracts from Turmeric and Persian lilac**

The plants were collected and washed for the first time with tap water. The second wash was conducted by sterile distilled water and then dried under shade in airy condition away from direct light. Dried plants were converted to the powder using a milling machine.

For each 100 mL of water, 10 g of turmeric/Persian lilac powder were added to the sterile distilled water and placed on a shaker at 100 rpm for 24 h. Then, the mixture of water and plant powder were clean up with a filter paper on a Buckner funnel using a vacuum pump. In order to change the plant extract to the powder, the obtained liquid was placed in the oven at 45 °C for 24 h. After water evaporation, the remaining powder were collected and used to prepare a solution with 100 mg/mL concentration. The solutions were kept at 4 °C until use (Azimi et al. 2006; Mariita et al. 2011; Alo et al. 2012).

**Chemical fungicide mixture**

We used a chemical mixture including Mancozeb (Dithane M-45®) 64% W/W as a non-systemic fungicide with protective action on contact and Metalaxyl (Ridomil®) 8% W/W with systemic function and inert ingredient 28% W/W, in order to compare its antifungal activity versus two plant extracts and thanatin. The fungicides were purchase from Al-Mahmood Company (Manama, Bahrain).

**Fungal isolates**

Fungal isolates were provided by the fungal collection at department of plant protection, Ferdwosi University of Mashhad as listed in Table 1.

**Antifungal activity test**

The plant extracts were mixed with medium in order to evaluate their antifungal activity. After preliminary estimation, the following concentrations 0, 16.66 and 25 mg/mL were prepared. Sterile distilled water was used as a solvent to dilute the stock solutions and as a negative control in treatments as well. PDA (Potato Dextrose Agar, Merck, Germany) was used as a growth medium for fungal cultivation. The medium was prepared, autoclaved and let to be cool down until 45 °C at room temperature. The plant extracts were added to the petri dishes with 6 cm diameter and mixed to prepare a uniform solution.
for each plant extract concentrations. The fungal disks with 6 mm diameter were taken from the 1 week fungal culture using a sterile cork borer and placed in the middle of petri dishes. The petri dishes were incubated at 28 °C and the growth rate of fungi were measured every 24 h until the fungal mycelia were completely occupied the surface of PDA in control plates. Three replications for each concentration were considered for all fungal isolates (Hadian et al. 2006). The same procedures were performed in the plates amended with thanatin and chemical mixture with different concentrations. According to preliminary tests, we decided to use the following concentrations of thanatin; 0, 0.264 and 0.400 µg/mL and the concentration of chemical mixture were; 0, 0.001, 0.01 and 0.1 mg/mL. Thus, the required concentrations of thanatin and chemical fungicide were added to the medium. Subsequently, the rate of mycelial growth inhibition was calculated according to the following equation (Moslem and El-Kholie 2009):

\[
MGI = \frac{(DC - DT)}{DC} \times 100
\]

where, MGI: mycelial growth inhibition rate (%), DC: diameter of the control samples, DT: diameter of the test samples.

**Determination of MIC and MFC**

The broth micro-dilution method (Irkin and Korukluoglu 2007; Plodpai et al. 2013) with some modifications was used to determine minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of different plant extracts, thanatin and chemical mixture. Accordingly, a serial dilution was prepared from the stock solution of different treatments. Sterile distilled water was used as a solvent to dilute the stock solutions. PDB (Potato Dextrose Broth, Merck, Germany) was prepared, autoclaved and used as a basic medium for MIC/MFC evaluation. Then, the fungal disks were prepared using a cork borer from the marginal part of 7 days old fungal colonies and placed into the sterile empty test tubes containing 5 mL of PDB. One millilitre of different plant extracts, thanatin and chemical mixture at different concentrations were taken and added into the test tubes and mixed. The tubes were incubated at 25 °C for 5 days. Positive control tubes containing only broth media and fungal disk as well as negative control tubes containing broth media accompanied by 1 mL sterile distilled water was prepared and incubated at the same conditions. The MIC was defined as the lowest concentration of treatments that inhibited the visible growth of the fungi. In order to determine the MFC, the fungal disks were removed from the test tubes and sub-cultured on Petri dishes containing PDA medium and incubated at 25 °C for 5 days to determine if the inhibition was reversible. The MFC was the lowest concentration that did not permit growth on the plates.

**In vivo antifungal activity test**

Tomato seedlings were used as host plants in order to investigate the potential efficacy of thanatin compared to the other material including plant extracts and chemical mixture. The plant pathogenic fungus *Alternaria tenuissima* causing early blight in tomato was selected as a test fungus.

The antifungal activities of different treatments were determined by a whole plant method in the greenhouse, as previously described by Bajpai and Kang 2010; Nashva and Abo-ElyouSr 2012). Briefly, the 7 week old tomato plants possessing an average of 3–5 leaves were kept in the green house with 14 h photoperiod and 60% humidity. The average temperature during day and night were 27 °C and 19 °C, respectively. The initial concentration of the test solutions were 1.6 µg/mL for thanatin, 100 mg/mL for both plant extracts and 2 mg/mL for chemical mixture. To prepare the test solutions, the stock solutions was diluted in 5 mL dimethylsulfoxide (DMSO) followed by dilution with the water containing Tween-20 (250 µg/mL). 30 mL from each solutions with different concentrations which were 1.6 and 0.8 µg/mL for thanatin, 25 and 50 mg/mL for both plant extracts and 1.5 and 2 mg/mL for chemical mixture, were individually sprayed as foliar application on tomato plants and repeated 15 days later. Two days after the second spraying, tomato plants were inoculated with 20 mL of A. tenuissima spore suspension containing 5 × 10⁶ spore/mL. After inoculation, plants were kept in a climate chamber at a daily temperature of 28 °C and 85% relative humidity. Disease development was recorded 15 days after inoculation. The severity of early blight was recorded for each treatment and scored from 0 to 9 according to the following rating system (0 = healthy plant, 1 = 1–5%, 3 = 6–10%, 5 = 11–25%,

### Table 1 Phytopathogenic fungi used in antifungal activity test

| Name                        | Strain number | Isolated host | Disease                   |
|-----------------------------|---------------|---------------|---------------------------|
| Geotrichum candididum       | IBRC-M 30010<sup>a</sup> | Potato        | Potato rot in storage     |
| Botrytis cinerea            | IBRC-M 30162<sup>b</sup> | Banana        | Grey mold rot            |
| Rhizoctonia solani          | IRAN 2957<sup>c</sup> | Potato        | Stem canker              |
| Alternaria tenuissima       | Local isolate | Tomato        | Early blight              |
| Gibberella fujikuroi        | NBRC 9976<sup>c</sup> | Rice          | Bakanae disease          |

<sup>a</sup> Iranian Biological Resources Center  
<sup>b</sup> Iranian Fungal Culture Collection  
<sup>c</sup> NITE Biological Resource Center
7 = 26–50% and 9 ≥ 51% of the leaf area infected) suggested by Latha et al. (2009). In vivo experiments were repeated twice.

**Data analysis**

All the analyses were performed in triplicate and datasets were subjected to analysis of variance (ANOVA) and the Duncan’s multiple range test using SPSS 24 software. In all cases, a P value of ≤ 0.05 was considered significant. The diagrams were drawn using Microsoft Office Excel 2013.

**Results**

**Transfected cell culture, thanatin expression and SDS-PAGE analysis**

Cultivation of transfected HEK293 cells and expression of recombinant thanatin were successfully accomplished. A 2.34 kDa band corresponding to the size of thanatin and industrial thanatin was observed on the SDS-PAGE, confirming that the peptide was properly expressed in the host cells and secreted into medium (Fig. 1) as documented by Fehlbaum et al. (1996) and Koch et al. (2012). The pcDNA3.1+ vector without thanatin encoding sequence was transfected into HEK 293 cell as a negative control and the total secretion protein of this cell was run alongside the transfected sample carrying thanatin coding sequence. As observed there was no band with such a size in the control sample.

**Antifungal activity test**

The results of antifungal effects and mycelial growth inhibition for turmeric, Persian lilac, chemical mixture and recombinant thanatin against all tested fungi have been presented in Figs. 2, 3, 4, 5 and 6, respectively. As shown turmeric has inhibited the growth rate of all tested fungi compared to the control samples in both applied concentrations (Fig. 2). The results of the Duncan test showed that, there is a significant difference between inhibition rates of *B. cinerea* compared to the other species. Basically, a higher rate of inhibition was observed in the higher application of turmeric extract in tested fungi. Turmeric at the concentration of 25 mg/mL had the highest amount of inhibition rate against *B. cinerea*. The lowest rate of inhibition was observed in the fungus *G. candidum* at 16.66 mg/mL concentration (Fig. 3A).

As has been presented, Persian lilac was inhibited the mycelial growth of all tested fungi excluding *G. candidum*, compared to the control samples in the applied concentrations (Fig. 4). In overall, the results of Duncan test showed that, higher rate of inhibition was observed in the higher concentration of Persian lilac extract in all tested fungi. The concentration of 25 mg/mL had shown

![Fig. 2 Antifungal effects of turmeric extract against different plant pathogenic fungi under in vitro condition](image-url)
Fig. 3  Mycelial growth inhibition of different fungal plant pathogen pretreated with turmeric extract (A), Persian lilac extract (B), chemical mixture contained Mancozeb + Metalaxyl (C) and recombinant thanatin (D)
the highest amount of inhibition rate against *B. cinerea*. There is a significant difference between the inhibition rates of *B. cinerea* compared to the other species. At 25 mg/mL concentration of Persian lilac, the fungi *A. tenuissima* and *R. solani* had shown no significant differences. The fungi *R. solani* and *G. fujikuroi* at 16.66 mg/mL concentration did not show any significant difference (Fig. 3B).

Regarding to the plant extracts, a simple comparison between Fig. 3A, B is showing that, turmeric has been found to be more effective than Persian lilac against tested fungi in similar concentrations.

As expected, chemical mixture was inhibited the mycelial growth of all tested fungi compared to the control samples in applied concentrations (Fig. 5). The results of Duncan test on chemical mixture indicated that, all treatments were significantly different in applied concentration. Obviously, by increasing of fungicide concentration, the rate of fungal inhibition will also be increased (Fig. 6). Interestingly, the applied concentrations of thanatin are not comparable with other three treatments. The highest and lowest inhibition rates were observed in the fungi *B. cinerea* and *G. candidum*, respectively (Fig. 3D).

**MIC and MFC results**

The minimum inhibitory concentration and minimum fungicidal concentration of different treatments were determined for the five plant pathogenic fungi (Table 2). As presented, both plant extracts and thanatin have shown inhibitory effects against all tested fungi. Remarkably, the concentrations of MIC and MFC are showing that, the antifungal effects of thanatin are much stronger than that of the plant extracts and chemical mixture too. The results are clearly showing that, thanatin is more potent than the other treatments in controlling five fungal plant pathogens.

**Antifungal evaluation under in vivo condition**

According to the results given in Fig. 7, the plant extract were shown a sensible antifungal activity under in vivo condition. Both plant extracts, significantly reduced the severity of fungal disease tomato early blight caused by *A. tenuissima* in applied concentrations (Fig. 8). The most effective treatments between plant extracts was turmeric
at 50 mg/mL concentration. Among all treatments, thanatin presented a good controlling effects on tomato early blight under in vivo condition (Fig. 8). Notably, the inhibition effect of thanatin was observed by its application at µg level, while the inhibitory effects of plant extracts were observed by their application at mg level.

Discussion
Human food security is threatened by different plant diseases and the quality and quantity of crops are reduced by several plant diseases which caused by diverse fungi, bacteria, nematodes, viruses and, etc. Amongst different plant pathogens, fungi are the most important organisms caused plant diseases so that, their control is unavoidable. The most commonly used method for inhibiting plant fungal pathogens is the application of chemical fungicides that are considered as environmental pollutants and also as potential threats to human health and environment (Damalas and Eleftherohorinos 2011; Andersson et al. 2014). Furthermore, plant pathogens are getting resistant to many of these chemicals fungicides (Hahn 2014). Consequently, the attention of many researchers has been drawn to the other alternatives and harmless materials with antifungal activity. For example, several studies have been accomplished on the application of essential oil and plant extracts to inhibit fungal plant pathogens (Sales et al. 2016).

In the present study, the antifungal activity of two plant extracts named turmeric and Persian lilac along with a chemical mixture and recombinant thanatin were evaluated against following fungal plant pathogens; G. candidum, B. cinerea, R. solani, A. tenuissima and G. fujikuroi. The obtained result confirmed the antifungal activity of all treatments against tested fungi. Between two plant extracts, turmeric showed a higher rate of mycelial inhibition against tested fungi than Persian lilac. Although,
the antifungal activity of plant extracts was satisfactory, the evaluated MIC and MFC values for them were much higher than that of chemical mixture which means two plant extracts were basically not comparable with chemical mixture (Table 2). However, in terms of maintaining health and environmental aspects, they are much more preferable than chemical fungicides. As a result, the aqueous extracts of turmeric and Persian lilac can be considered as good alternatives to control fungal plant disease as previously documented by Damalas 2011 in case of turmeric.

Another suggestion is to utilize from the potential of antimicrobial peptides (Datta et al. 2015). In this regard, thanatin is one of the most appropriate antimicrobial peptides that we would like to offer as a promising compounds to control fungal plant diseases. The valuable features of thanatin, such as being non-allergic for human (Wu et al. 2011) made this peptide encoding sequence as a good candidate to be insert in plants for making transgenic resistance crops against fungal pathogens (Wu et al. 2010, 2013; Imamura et al. 2010, 2016). However, due to the limitations and disadvantages of transgenic plants (Lu 2008; Prakash et al. 2011), synthetizing of thanatin in the form of recombinant and secretion in HEK293 can be more efficient and manageable in the wide scale. It should also be mentioned that, the cost of production for this antimicrobial peptide is not comparable to chemical fungicides. Regarding to the great antifungal and antibacterial (Mamarabadi et al. unpublished data) activity of thanatin and its compatibility with human health and

---

**Table 2** Determination of MIC and MFC for different treatments tested against five fungal plant pathogen

| Fungi         | A. tenuissima | R. solani | B. cinerea | G. fujikuroi | G. candidum |
|---------------|---------------|-----------|------------|--------------|-------------|
| MIC/MFC (µg/mL) | MIC MFC      | MIC MFC   | MIC MFC    | MIC MFC      | MIC MFC     |
| Turmeric      | 6250 50,000   | 3250 25,000| 3125 25,000| 6250 50,000  | 12,500 100,000|
| Persian lilac | 25,000 –      | 12,500 –  | 12,500 –   | 25,000 –     | 50,000 –    |
| Chemical mixture | 500 2000 | 500 2000  | 250 1000   | 250 1000     | 500 1500   |
| Thanatin      | 0.6 1.2       | 0.6 1.2   | 0.14 0.6   | 0.3 0.6      | 1.2 2.4     |

---

![Antifungal effects of recombinant thanatin against different plant pathogenic fungi under in vitro condition](image)
environmental safety compared to unpleasant effects of chemical fungicides, we could imagine a clear perspective for this peptide with the intention of its application in sustainable agriculture.

Additional file

Additional file 1. Schematic representation of thanatin nucleotide and amino acid sequences.
Authors' contributions
MM conceived and supervised the project. AT designed the experiments and wrote the manuscript. YR performed the experiments and analyzed the data. All authors read and approved the final manuscript.

Author details
1 Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashahad, Iran. 2 Department of Biotechnology and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashahad, Iran.

Acknowledgements
We thank Ms. Marjan Azghandi for her technical assistance.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
All data are presented in figures and tables within this article. Any material used in this study will be available for research purposes upon request.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This article does not contain any studies with human participants or animals performed by any of the authors.

Funding
This study was funded by the deputy of research and technology, Ferdowsi University of Mashhad, Iran (Grant No: 45239).

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 27 May 2018 Accepted: 29 October 2018
Published online: 02 November 2018

References
Aggarwal BR, Sundaram C, Malani N, Ichikawa H (2007) Curcumin: the Indian solid gold. The molecular targets and therapeutic uses of curcumin in health and disease. Springer, Boston, pp 1–75
Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS (2012) Antibacterial activity of water, ethanol and methanol extracts of Ocimum gratissimum, Vernonia amygdalina and Afromomum melegueta. Adv Appl Sci Res 3:844–848
Andersson H, Tago D, Treich N (2014) Festicides and health: a review of evidence on health effects, valuation of risks, and benefit-cost analysis. preference measurement in health, vol 5. Emerald Group Publishing Limited, Bingley, pp 203–295
Andow DA, Zwahlen C (2006) Assessing environmental risks of transgenic plants. Ecol Lett 9:196–214. https://doi.org/10.1111/j.1461-0248.2005.00846.x
Avancio GB, Ferreira FD, Bomfim NS, Peralta RM, Brugnari T, Mallmann CA, Andersson H, Tago D, Treich N. (2014) Festicides and health: a review of evidence on health effects, valuation of risks, and benefit-cost analysis. Springer, Boston, pp 203–295
Awana MG, Ntshangase P, Bava AS, Sitas F, Field D, Nkemdirim J (2018) Prevention of outdoor air pollution: a review of medicinal plant use in South Africa. Front Public Health 6:97. https://doi.org/10.3389/fpubh.2018.00097
Azmi AA, Delnavaz HB, Mansour GA (2008) Antifungal effect of aqueous alcoholic and phenolic extracts of seed and leaves of Sorghum bicolor against Fusarium solani Fusarium poa in Persian. Med Plant 6:26–32
Bajpai VK, Kang SC (2010) Antifungal activity of leaf essential oil and extracts of Metasequoia glyptostroboides Miki ex H. J Am Oil Chem Soc 87:327–336. https://doi.org/10.1007/s11746-009-1500-6
Carvalho FP (2017) Festicides, environment, and food safety. Food and Energy Secur 6:48–60. https://doi.org/10.1007/978-3-319-68239-1_4
Damalas CA (2011) Potential uses of turmeric (Curcuma longa) products as alternative means of pest management in crop production. Plant Omics 4:136

Mammarabadi et al. AMB Expr (2018) 8:180

Damalas CA, Eleftherohorinos IG (2011) Festicide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8:1402–1419. https://doi.org/10.3390/ijerph8051402
Datta A, Ghosh A, Airoldi C, Sperandeo P, Mroue KH, Jiménez-Barbero J, Kundu P, Ramamoorthy A, Bhunia A (2015) Antimicrobial peptides: insights into membrane permeabilization, lipopoly saccharide fragmentation and application in plant disease control. Sci Rep 5:11951. https://doi.org/10.1038/srep11951
Fehlbauern B, Bulet P, Chemshy S, Briend JP, Roussel JP, Letellier L, Huet C, Hoffmann JA (1996) Structure-activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides. Proc Natl Acad Sci 93:1221–1225
Ferreira FD, Mossini SA, Ferreira FM, Arrotéia CC, da Costa CL, Nakamura CV, Machinski Junior M (2013) The inhibitory effects of Curcuma longa L. essential oil and curcumin on Aspergillus flavus link growth and morphology. Sci World J. https://doi.org/10.1155/2013/343804
Hadjan J, Falah T, Ghorbanipour M, Salehi P, Haji EB (2008) A phytochemical study of Cymbopogon parkeri stapf. Essential oil, and ITS biological activity against some phytopathogenic fungi. Iran J Agric Sci 37:425–431
Hafidh RR, Abdulamir AS, Vern LS, Bakar FA, Abbas F, Jahanshie F, Sekawi Z (2011) Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. Open Microbiol J 5:96–106. https://doi.org/10.2174/1874285801105010096
Hahn M (2014) The rising threat of fungicide resistance in plant pathogenic fungi: Batraxis as a case study. J Chem Biol 7:133–141. https://doi.org/10.1016/j.jchbiol.2015.12.006
Hu J, Han J, Li H, Zhang X, Lan LL, Chen F, Zeng B (2018) Human embryonic kidney 293 cells: A vehicle for biopharmaceutical manufacturing, structural biology, and electrophysiology. Cells Tissues Organs 205:1–8. https://doi.org/10.1159/000485501
Imamura T, Yasuda M, Kusano H, Ohno Y, Kamakura T, Taguchi S, Shimada H (2010) Acquired resistance to the rice blast in transgenic rice accumulating the antimicrobial peptide thanatin. Transgenic Res 19:415–424. https://doi.org/10.1007/s11248-009-9320-x
Imamura T, Sekine KT, Tamahata T, Kusano H, Shimada H (2016) Production of recombinant thanatin in wheat rice seeds that lack an accumulation of storage starch and proteins. J Biotechnol 219:28–33. https://doi.org/10.1016/j.jbiotec.2015.12.006
Irkin R, Koruklugul M (2007) Control of Aspergillus niger with garlic, onion and leek extracts. Afr J Biotechnol 6:1–6. https://doi.org/10.1155/2013/343804
Keymanesh K, Sohtan S, Sardani S (2009) Application of antimicrobial peptides in agriculture and food industry. World J Microbiol Biotechnol 25:933–944. https://doi.org/10.1007/s11274-009-9984-7
Koch A, Khalifa W, Langen G, Vilcinskas A, Kogel KH, Imani J (2012) The antibiotic activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani. Biol Control 50:85–93. https://doi.org/10.1016/j.biocontrol.2009.03.002
Lu BR (2008) Transgene escape from GM crops and potential biosafety consequences: an environmental perspective. Collect Biosaf Rev 4:66–141
Mandard N, Sodano P, Labbe H, Bonmatin JM, Bulet P, Hetru C, Prat-M, Vovelle F (1998) Solution structure of thanatin, a potent bactericidal and fungicidal insect peptide, determined from proton two-dimensional nuclear magnetic resonance data. Eur J Biochem 256:404–410
Marita RM, Ogol CK, Oguge NO, Okerno PD (2011) Methanol extract of three medicinal plants from samburu in northern kenya show significant antimycobacterial, antibacterial and antifungal properties. Res J Med Plants 5:54–64
Martinez-Correia HA, Paula JT, Kayano AC, Queiroga CL, Magalhães PM, Costa FT, Cabral FA (2017) Composition and antimicrobial activity of extracts of Curcuma longa L. obtained by a combination of extraction processes using supercritical CO2, ethanol and water as solvents. J Supercrit Fluids 119:122–129
Montesinos E (2007) Antimicrobial peptides and plant disease control. FEMS Microbiol Lett 270:1–11. https://doi.org/10.1111/j.1574-6968.2007.00683.x

Moslem MA, El-Kholie EM (2009) Effect of neem (Azadirachta indica A. Juss) seeds and leaves extract on some plant pathogenic fungi. Pak J Biol Sci 12:1045

Nashva SM, Abo-ElyouSr KA (2012) Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Prot Sci 48:74–79

Neycee MA, Nematzadeh GH, Dehestani A, Alavi M (2012) Assessment of antifungal effects of shoot extracts in chinaberry (Melia azedarach) against S phyltopathogenic fungi. Int J Agric Crop Sci 4:474–477

Pingali PL, Roger PA (eds) (2012) Impact of pesticides on farmer health and the rice environment, vol 7. Springer, Berlin

Plodpai P, Chuenchitt S, Petcharat V, Chakthong S, Voravuthikunchai SP (2013) Anti-Rhizoctonia solani activity by Desmos chinensis extracts and its mechanism of action. Crop Prot 43:65–71. https://doi.org/10.1016/j.croprot.2012.09.004

Prakash D, Verma S, Bhatia R, Tiwary BN (2011) Risks and precautions of genetically modified organisms. ISRN Ecol. https://doi.org/10.5402/2011/369573

Saleem R, Rani R, Ahmed M, Sadaf F, Ahmad SI, ul Zafar N, Khan SS, Siddiqui BS, Ansari F, Khan SA, Faizi S (2008) Effect of cream containing Melia azedarach flowers on skin diseases in children. Phytomedicine 15:231–236. https://doi.org/10.1016/j.phymed.2008.02.002

Sales MD, Costa HB, Fernandes PM, Ventura JA, Meira DD (2016) Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pac J Trop Biomed 6:26–31. https://doi.org/10.1016/j.apjtb.2015.09.026

Sen A, Batra A (2012) Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res 4:67–73

Stenberg JA (2017) A conceptual framework for integrated pest management. Trends Plant Sci 22:759–769. https://doi.org/10.1016/j.tplants.2017.06.010

Tanhaeian A, Shahriari Ahmadi F, Sehkavati MH, Mamarabadi M (2018) Expression and purification of the main component contained in camel milk and its antimicrobial activities against bacterial plant pathogens. Probiotics Antimicro Prot 10:787–793. https://doi.org/10.1007/s12602-018-9416-9

Thomas P, Smart TG (2005) HEK293 cell line: a vehicle for the expression of recombinant proteins. J Pharmacol Toxicol Methods 51:187–200. https://doi.org/10.1016/j.vascn.2004.08.014

Wolfenbarger LL, Phifer PR (2000) The ecological risks and benefits of genetically engineered plants. Science 290:2088–2093. https://doi.org/10.1126/science.290.5499.2088

Wu G, Wu H, Li L, Fan X, Ding J, Li X, Xi T, Shen Z (2010) Membrane aggregation and perturbation induced by antimicrobial peptide of S-thanatin. Biochem Biophys Res Commun 395:31–35. https://doi.org/10.1016/j.bbrc.2010.03.107

Wu G, Li X, Fan X, Wu H, Wang S, Shen Z, Xi T (2011) The activity of antimicrobial peptide S-thanatin is independent on multidrug-resistant spectrum of bacteria. Peptides 32:1139–1145. https://doi.org/10.1016/j.peptides.2011.03.019

Wu T, Tang D, Chen W, Huang H, Wang R, Chen Y (2013) Expression of antimicrobial peptides thanatin (S) in transgenic Arabidopsis enhanced resistance to phytopathogenic fungi and bacteria. Gene 527:235–242. https://doi.org/10.1016/j.gene.2013.06.037

Zasloff M (2002) Antimicrobial peptides of multicellular organisms. Nature 415:389. https://doi.org/10.1038/415389a