



Research Article

Effective Management of Ginger Soft Rot Caused by *Penicillium digitatum* through Plant Extracts

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Abstract | *In-vitro* experimentation consisting of crude extracts of different plants and fungicides were performed for determination of antifungal activity against *Penicillium digitatum*, a plant pathogenic fungi responsible for ginger soft rot. Petri dish were used to conduct bioassay through poisoned food technique with triplicates. The crude extract of all the plants showed a better inhibitory effects on the pathogen's (*P. digitatum*) mycelial growth rather than fungicides. Among all the plant extracts, the best result was showed by garlic extract with 87% of mycelial growth inhibition followed by moringa with 82%, the best concentration is 100mg/mL. Besides, Ridomil gold showed the most effective control against ginger soft rot with 84% of mycelial inhibition growth among all the tested fungicides. And found that protective treatment had a best effect than curative treatment. These results revealed that plants are rich source of metabolites with a wide range of antifungal activity against ginger soft rot. Crude extracts of these plants have potential to become effective biopesticides upon purified fractions with no hazardous effects on environment as well as humans.

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Keywords | Ginger soft rot, *Penicillium digitatum*, Biopesticides, Plant extract, Antifungal activity

Introduction

Ginger (*Zingiber officinale* Rosc.) is a perennial, herbaceous rhizomic vegetable having great importance in medicine and spice. The export and import value of ginger is globally enhancing every year, whereas soft rot causes significant yield losses, which ranges from 15% to 50% (Indo-Swiss Project Sikkim, 2005; Behera *et al.*, 2020), manage the disease is very important for ginger production.

Penicillium digitatum as the pathogen of ginger soft rot has been found for the first time. Phyto-insecticides gain more popularity in the market due to their multi active services (Isman, 2014). For instance, commercial plant products including pyrethrum have been known effective against insect pests because they retain the neurotoxicant effects which is responsible for knock down and paralysis resulting in insects mortality (Grdiša and Gršić, 2013). Phyto-pesticides are also responsible for disturbance in

significant enzymes production, some of them causes moulting resulting in inhibition of mycelial growth and pathogen development (Ntalli and Menkissoglu-Spiroudi, 2011). Some plant extracts including turmeric and garlic (*Curcuma longa* and *Allium sativum*) are found to be effective against red flour beetle (*Tribolium castaneum*) as they are responsible for causing repellence, poisonousness, mortality and inhibition of offspring emergence (Ali *et al.*, 2014).

Major symptoms of ginger soft rot yellowing of leaves and stem, stunted growth, brown discoloration of water-conducting tissues, root rotting with black discoloration which ultimately leading to rotting of rhizome (Golliker and Brown, 1972; Mekbib, 2007; Mohana and Raveesha, 2007; Youssef *et al.*, 2011; Sanzani *et al.*, 2014). The optimum temperature for rapid development of soft rot is 24°C while below 10°C and above 30°C grow slowly (Mekbib, 2007).

Ginger soft rot can be control by synthetic fungicide effectively but it also shows a bad effect on the environment, human as well as other organisms. Plant extract is an alternative non-chemical pesticide due to its non-toxic effect, availability and eco-friendly nature. Plant extract has great antimicrobial and cytotoxic effects on microorganism's e.g. chemical of mustard allyl isothiocyanate is capable to reduce the growth of *P. expansum* (Ikeura *et al.*, 2011; Ali *et al.*, 2018).

The main purpose of this work is to manage (*P. digitatum*) on ginger by using various plant extract and their comparison with fungicidal control.

Materials and Methods

Sample collection and isolation

Ginger with symptoms of fungal infection was collected from the local market and cold storages of Bahawalpur District and brought to the Plant Pathology Laboratory at University College of Agriculture & Environmental Sciences (The Islamia University of Bahawalpur). The samples were washed with tap water, surface sterilized for two minutes with 2% Clorox and then rinsed with sterilize distilled water (SDW) for three times. Slices of infected tissues ranging from three to five millimeter were cut from the margins with sterile scalpel and lodged in petri plates having (15 mL/plate) PDA (potato dextrose agar) medium, then incubated at 28°C for 5 days under 12 h photoperiod. Hyphal tip

method was used for purification of the fungus (Tuttle, 1969) and the purified isolates were preserved on PDA slants for further use.

Pathogenicity test

Wound inoculation method was followed to confirm the pathogenicity of isolated fungi in laboratory (Moalemiyan *et al.*, 2007). Healthy gingers were washed with water and surface sterilized by 5% NaOCl for 60 sec. following 3 times rinsing with SDW. Fungus spore suspension was prepared from 7 days old culture by mixing 15 mL SDW in petri plates. Injuries were made on gingers with sterilized needle and inoculated with 10⁵ spores/mL, untreated gingers served as controlled and incubated at 28°C for 7 days. To confirm the pathogenicity (Koch, 1876), *P. digitatum* was re-isolated from the infected part and characterized to confirm its pathogenicity.

Preparation of aqueous extracts

In-vitro extract from leaves of four plants viz. Garlic (*Allium sativum*), Sohanjna (*Moringa oleifera*), Neem (*Azadirachta indica*) and Akk (*Calotropis gigantea*) were collected from the various parts of Bahawalpur District. Healthy and fresh leaves of plants (50 g) washed thoroughly and crushed by adding 50 mL of SDW in waring grinder for 10 min. The resultant saturate was dribbled through two layers of muslin cloth and then the extract was centrifuged at 4000 rpm for 30 min. The filtrate was strained through filter polycarbonate membrane 0.22 mm, and then ethyl acetate was used twice for the extraction of the filtrate and the final crude extract was obtained after drying the filtrate in a vacuum (Shakeel *et al.*, 2016). The extract was aseptically conserved at 4°C for further use (Satish *et al.*, 1999). The prepared extract was termed as (100% concentration) crude extract.

Preparation of plant extract dilutions

For the preparation of dilutions of all the plant extracts, 100 mg of plant extracts was dissolved in 1 mL of SDW and further diluted by serial dilution method to obtain required dilutions i.e. 12.5mg/mL, 25mg/mL, 50mg/mL. The inoculation volume was 0.5 mL/plate containing 20 mL of media while the control treatment remained as it. There were five treatments (including control) and three replications of each treatment.

In-vitro management of *P. digitatum* using fungicides

Fungicides namely Ridomil gold, Bio magic and

Anthem were purchased from the local market to evaluate their efficiency on growth of mycelial diameter of *P. digitatum* by Poisoned food technique (Grower and Moore, 1962; Falck, 1907). Three different concentration of fungicides viz Ridomil gold (Mancozaib 64% w/w), Bio magic (*Bacillus subtilis* 10 million CFU/g) and Anthem (Pyroxasulfone 22.61% w/w) were distributed individually in sterilized molten PDA medium. 0.25 mg, 0.5 mg and 1 mg of fungicides were dissolved in SDW to make 250, 500 ppm and 1,000 ppm concentration. Petri dishes were incubated at 28°C for 5 days. The growth diameter of targetd pathogen was measeured and compared with control to inhibition percentage of fungicides.

Assesment of treatment time effect of various plant extract and fungicides on penecillium digitatum disease index

The examined constituents included 0.5 mL of aqueous solution of garlic and moringa with 200 mg/mL while Ridomil gold and Anthem with 2,000 mg/mL. The treatments were: (i) the control in which ginger was not treated with any treatment. (ii) the protective treatments in which ginger was inoculated individually with plant extract and fungicides 24 h before the inoculation of *P. digitatum* (iii) the competition treatments in which ginger was inoculated individually with plant extract and fungicides along with *P. digitatum* at the same time. (iv) the curative treatments in which ginger was inoculated individually with plant extract and fungicides 24 h after the inoculation of *P. digitatum* (Shakeel *et al.*, 2016). Each treatment has three replications.

Comparison between effect of plant extract and fungicides

The two most effective plant extract viz garlic and moringa and the two most effective fungicides namely Ridomil gold and Anthem were selected and experiment was made for their comparison to select the best one among all. Different concentrations (50, 100 and 200 mg/mL) were made for plant extract while in case of fungicides 500 ppm, 1,000 ppm and 2,000 ppm concentration were made for the experiment. Each treatment has three replications.

Preparation of poisoned food plates

For inhibition of mycelial growth Poisoned Food Technique was used in Potato Dextrose Agar (Groover and Moore, 1962; Shahi *et al.*, 1999). After autoclaving the PDA it was retained at 40°C in a water bath. The prepared extracts and fungicides were individually poured into sterilized molten PDA in

different concentrations. The medium was transferred into petri plates of 60×15mm. Mycelial discs of 5 mm were transferred in the center of all petri plates after solidification of the media. The discs were taken from the mature culture plate i.e., one week old culture of the pathogen. There were eight treatments including control and there was three replications of each treatment. Controlled petri dishes remained untreated.

Petri dishes were incubated at 28°C for 7 days. Untreated Dishes were filled with mycelial growth. (Groover and Moore, 1962; Fang, 1998). After incubation, the mycelial growth diameter of *P. digitatum* was measured in mm (Singh and Tripathi, 1999). Regarding inhibition percentage of pathogenic growth and the antifungal activity of the extracts and fungicides were computed through the given formula:

$$\text{Inhibition percent} = \frac{c - T}{c} \times 100$$

Where; C= Average increase of fungal diameter in control and T= Average increase of fungal diameter in treatment (Mohana and Raveesha, 2007).

Due to the treatment of different fungicides at different concentration the percent inhibition in growth was calculated as follows:

$$\text{Inhibition of mycelial growth (\%)} = \frac{dc - dt}{dc} \times 100$$

Where; dc= average of fungal mycelial diameter in control, and dt= average of fungal mycelial diameter in treatment (Taskeen-Un-Nisa *et al.*, 2011).

Statistical analysis

Data from *in-vitro* antifungal assays was exposed to an ANOVA study, followed by a least significance difference (LSD) test. Statistical analyses were performed with SAS/STAT Software for Windows.

Results and Discussion

Pathogenicity test

Symptoms started to appear after 24-36 h. Early symptoms showed slightly discolored spots. After 6-7 days, extensive green sporulation of fungus surrounded by a dense whitish band of mycelial growth was appeared on the surface of ginger. Slide

was prepared for the observation of hyphal structure of the fungus under the microscope. On taxonomic and morphological basis the pathogen was identified as *P. digitatum* that was the causal agent of soft rot.

Effecacy of plant extract regarding inhibition of mycelial growth

The antifungal activity of plant extract at various concentrations was evaluated through food poisoned method on PDA media. The result showed that the most effective mycelial inhibition percentage (87%) on PDA media amended with the garlic extract and moderate activity was observed in extract of Moringa (82%), Akk (41%) was found as low significant for mycelial inhibition and Neem (17%) showed least mycelial inhibition of *P. digitatum* (Figure 1). The LSD analysis of data revealed that aqueous extract of garlic significantly inhibited the hyphal growth and spore germination rate of the fungus. Inhibition percent of tested pathogen on PDA media showed that garlic was highly effective and Neem showed least effectiveness. The best control was observed at the concentration of 100mg/mL (Figure 2).



Figure 1: Comparison of control treatment with different plant extract for the inhibition of mycelial growth of *Penicillium digitatum* where; (a) garlic extract (100mg/mL), (b) Control plate (c) moringa extract (100mg/mL).

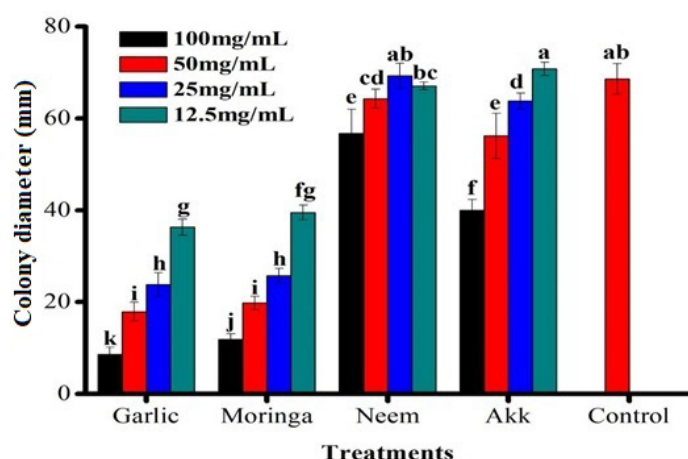


Figure 2: Effect of plant extract on the mycelial inhibition of *Penicillium digitatum*.

Effect of fungicides on the mycelial growth inhibition

The result revealed that highly significant percent inhibition (84%) of mycelial growth of *P. digitatum* was

observed in PDA media amended with the Ridomil gold fungicide and moderate activity was observed in Anthem fungicide (66%). The bio-fungicide Bio magic (30%) was found as least significant for mycelial inhibition of *P. digitatum* (Figure 3). The LSD analysis of the data revealed that ridomil gold significantly inhibited mycelial growth and spore germination of *P. digitatum*. Percent inhibition of tested fungi on PDA media revealed that ridomil gold was highly effective and Bio magic showed the least effectiveness (Figure 4).

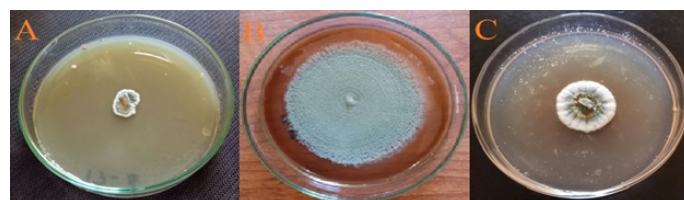


Figure 3: Comparison of control treatment with different fungicides for the inhibition of mycelial growth of *Penicillium digitatum* where; (a) Ridomil Gold (1000ppm), (b) Control plate and (c) Anthem (1000ppm).

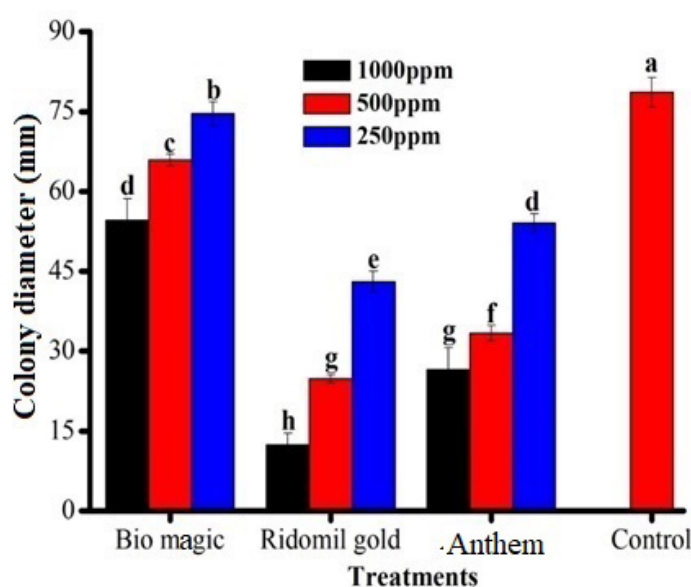


Figure 4: Effect of fungicides on the mycelial inhibition of *Penicillium digitatum*.

Assesment of treatment time effect of various plant extract and fungicides on Penicillium digitatum disease index

The time for treatment of plant extract and fungicides affected the mycelial growth inhibition of *P. digitatum* (Figure 5). The maximum inhibition in *P. digitatum* growth and development was observed in protective treatment. Competition treatment was the second best treatment regarding the reduction of *P. digitatum* while the curative treatment was the least effective. Among all plant extract and fungicides, garlic extract proved to be best for managing *P. digitatum* (Figure 5).

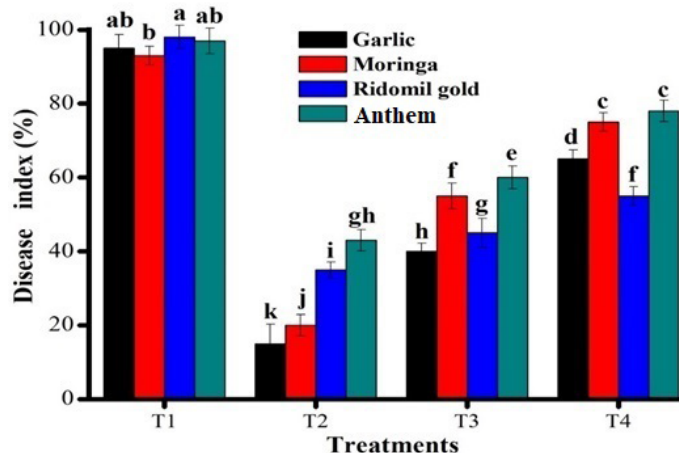


Figure 5: Assessment of effect of treatment time of different plant extract and fungicides on *Penicillium digitatum* disease index on ginger.

Comparison between effect of plant extract and fungicides
In the comparison of plant extract and fungicide, the garlic with the concentration of 200 mg/mL was the highly significant in the reduction of growth of *P. digitatum* (Figure 6). Moringa with the concentration 200 mg/mL and Ridomil gold fungicide with the concentration of 2,000 ppm was the 2nd best in controlling of *P. digitatum*. Anthem showed least mycelial inhibition of *P. digitatum* as compared to others (Figure 6).

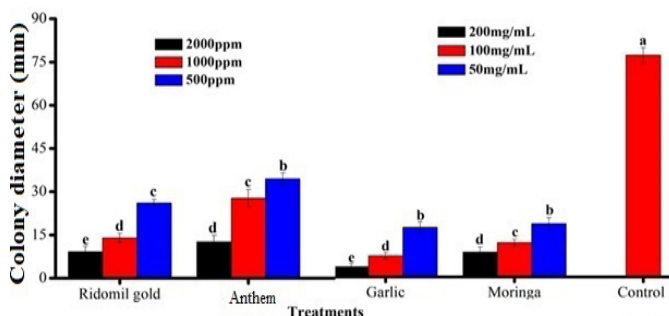


Figure 6: Comparison between effects of different plant extract and fungicides on the mycelial inhibition of *Penicillium digitatum* on ginger.

Crude extract of plant and fungicides were tested for their efficacy against *P. digitatum*. The efficiency of tested materials ranged from 5% to 90% in growth inhibition of *P. digitatum*. The main source of ginger soft rot is very common and undefined in their host range. It is a main issue of broad array of horticultural crops (Chaube and Pundhir, 2005; Onu et al., 2002). There is no single adequate method for the management of *P. digitatum* is available (Babadoost, 2004). Currently, synthetic fungicides are known to be the most successful technique of disease management. Although, there are lots

of concerns related to use of synthetic chemicals which makes them unfit to be considered in long term solution. Major concerns related to the use of synthetic chemicals are environmental pollution, public health problems, poisonous effect on non-targeted individuals, reduction in crop quality and above all resistance development in agricultural pests and disease causing agents (Kagala, 2004; Rai et al., 2006; Rahman et al., 2010). WHO has prohibited several effective pesticides because of their hazardous effect on environment as well as on humans (Barnard et al., 1997). Considering these limitations of synthetic pesticides, we are in a dear need to find alternatives for managing rhizome soft rot of ginger (Pandey et al., 2010). Most suitable, environmentally safe option instead of synthetic pesticides is use of natural plant products for managing phytopathogenic issues. Effectiveness of plant extract has already been reported in different studies (Sankarasubramanian et al., 2008; Mishra et al., 2009; Yanar et al., 2011; Talibi et al., 2012). Many researchers has reported the antifungal activity of certain plant extracts against various phyto-pathogens (Taskeen-un-Nisa et al., 2011; Amadioha, 2000; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005). Assessment of plant extract against other fungi responsible for causing rot i.e. *Pythium aphanidermatum* etc. has already been published under *in-vivo* and *in-vitro* conditions (Sagar et al., 2007; Haouala et al., 2008; Suleiman and Emua, 2009). Current investigation, different plant extract and fungicides with their inhibitory activity on other fungi were used. Present study results cleared revealed the effectiveness of different plant extract against *P. digitatum* under *in-vitro* condition. The encouraging mycelial inhibition percentage (around 90% in the present study) indicates that plant under consideration has wide fungicidal activity. For advance study of fractionation, basis could be formed for detecting active fractions. The current study was conducted to assess the different plant extracts to demonstrate the fact that the plants still serve as a reservoir of several pharmaceuticals that could be isolated and used against various plant diseases. It provides an eco-friendly alternative plant disease control method to chemicals.

Conclusions and Recommendations

Crude extract of plants are effective against *P. digitatum* which has potential to cause economic losses. These plant extracts which were showing effective results

have potential to be included in organic farming for development of effective biological fungicides. Garlic extract with 87% effectiveness proved to be lethal for management of disease following moringa extract (82%). The best concentration for their maximum management was 100 mg/mL. Moreover it was proved that prevention is better than cure as protective treatment was most effective among all treatments. These results revealed that plants are rich source of metabolites with a wide range of fungicidal activity against *P. digitatum*. The crude extract of these plants has potential to become effective biopesticides upon purified fractions with no hazardous effects on environment as well as humans. Furthermore, exploitations of natural plant extracts, which hinders the pathogenic growth, would be a more accurate and economical method to produce commercial pesticides for the protection of agricultural crops, with a major concern about plant disease management.

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Novelty Statement

This is first time that attempt has been made to address the post-harvest losses of ginger at market level.

Author's Contribution

Qaiser Shakeel: Designed and conducted experiments.

Rabia Tahir Bajwa: Conducted experiments.

Yasir Iftikhar: Supervised experiments.

Mustansar Mubeen: Designed and conducted experiments.

Muhammad Luqman: Statistical analysis.

Waqas Ashraf: Part of paper write-up.

Ifrah Rashid: Conducted experiments.

Conflict of interest

The authors have declared no conflict of interest.

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