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Effects of applying cold and hot aqueous extracts of ginger to control onion rot disease caused by *Aspergillus niger*

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ABSTRACT

Current study aimed to evaluate the efficiency of applying cold and hot aqueous ginger extracts to control onion rot disease caused by *Aspergillus niger*. We observed significant effect of the cold aqueous ginger extract on reducing the averaged *A. niger* growth on PDA culture medium and also the decreased average of onion spoilage when dipping in this extract as it exceeded hot aqueous extract. Results showed that there was an inverse relationship between the concentration of ginger and the growth of the pathogenic fungus either on PDA or the average of onion spoilage when it dipped in both extracts. The elevated concentration of ginger extract, decreased its effect on *A. niger* and vice versa, as the low concentration of ginger extract was significantly exceeded the high concentration in the reduction of the growth of pathogenic fungus when 5 g L^{-1} resulted in 5.10 and 5.20 cm reduction for cold and hot extracts respectively. Using 30 g L^{-1} exhibited a reduction in the growth of fungus amounted to 6.45 and 6.70 cm for cold and hot extracts respectively.

Key words: *Aspergillus niger*, onion, ginger, cold and hot aqueous extracts.
Article type: Research Article.

INTRODUCTION

Onion is an important vegetable plant belongs to Amaryllidaceae family and cultivated in the tropics and temperate regions of the world. It consists of a series of concentric swollen leaves that sit on a disk or short stalk. This crop comes after tomato economically as it is widely cultivated in Asia and Europe (Griffith *et al.* 2002). Onions are exposed to many plant diseases especially the regenerating fungi which enter through wounds and lead to a great loss when they grow in the field or the bulbs rot in the store (El-Nagerabi & Abdalla 2004). After harvesting, onions are infected with several diseases including black rot, blue rot, neck rot and brown rot, black and brown rot are among the diseases prevalent in the store (Raju & Naik 2007). The most devastating disease for onions in the field and storage is black rot (Tepe *at al.* 2004) which infects over 80% of storage bulbs (El-Nagerabi & Ahmed 2003). *Aspergillus niger* is the main cause of black rot disease on fruits and vegetables such as grapes, peanuts, onions and other food (Samson *et al.* 2001). The use of chemical fungicides to treat bulbs after harvesting causes health problems for consumers particularly some of these fungicides have toxic residues. Thus, the aim of this study was to use cold and hot aqueous ginger extract as environmentally friendly substance to control onion rot disease caused by *A. niger*.

MATERIALS AND METHODS

Preparation of the cold aqueous extract of ginger

The cold aqueous extract of ginger was prepared as 5, 10, 15, 20, 25 and 30 g L^{-1} by extracting each concentration in 250 mL of sterile distilled water for 24 h. The extract was filtered by piece of clothes in 1 L flask then the size was completed to 1 L. PDA was autoclaved for 20 min then 250 mg L^{-1} of chloramphenicol was added to inhibit

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the growth of bacteria. PDA and cold extract of ginger were mixed very well and poured into petri plates with an average of 15-20 mL for each plate and three replicates for each treatment. 0.5 cm disc from pure culture of *A. niger* was taken to the centre of each plate and when the radial growth of the fungus reached the end of plates area, the radial growth was measured by a ruler for two axis then the total divided by two (Shahbazi *et al.* 2015).

The hot aqueous extract of ginger

It was prepared using the same steps above but the difference was boiling ginger parts in 500 min distilled water for 20 min.

The effect of dipping the bulbs in cold aqueous extract of ginger

8 diameter bulbs of EWG cultivar were dipped in the cold aqueous extract of ginger for each 5, 15 and 30 g L^{-1} in 250 mL for 24 h. The bulbs were inoculated with 1.95×10^5 spore suspension of *A. niger* and the treatments were arranged as follows:

1- Distilled water only.
2- Spore suspension of *A. niger*.
3- Wounds only.
4- Spore suspension of *A. niger* + wounds.
5- Ginger (5 g L^{-1}).
6- Ginger (15 g L^{-1}).
7- Ginger (30 g L^{-1}).
8- Spore suspension of *A. niger* + Ginger (5 g L^{-1}).
9- Spore suspension of *A. niger* + Ginger (15 g L^{-1}).
10- Spore suspension of *A. niger* + Ginger (30 g L^{-1}).
11- Wounds + Ginger (5g L^{-1}).
12- Wounds + Ginger (15 g L^{-1}).
13- Wounds + Ginger (30 g L^{-1}).
14- Spore suspension of *A. niger* + Wounds + Ginger (5 g L^{-1}).
15- Spore suspension of *A. niger* + Wounds + Ginger (15 g L^{-1}).
16- Spore suspension of *A. niger* + Wounds + Ginger (30 g L^{-1}).

Each experiment was done with three replicates and each replicate was placed in 1 kg plastic bag in room temperature 25 ºC then results of pathogen damages on bulbs were recorded every 7 days until it spoiled in the positive control (Spore suspension of *A. niger* + Wounds).

The effect of dipping the bulbs in hot aqueous extract of ginger

8 diameter bulbs of EWG cultivar were dipped in the hot aqueous extract of ginger for each 5, 15 and 30 g L^{-1} concentrations in 250 mL for 24 h. The bulbs were inoculated with 1.95×10^5 spore suspension of *A. niger* and the treatments were arranged as mentioned above.

Statistical analysis

All experiments were conducted using complete randomize design (CRD) and means were compared with least significant difference (L.S.D) at 0.05. GenStat (12^{th} edition) program was used to analyse the data.

RESULTS AND DISCUSSION

Results of Table 1, Figs. 1 and 2 showed that both aqueous extracts (cold and hot) of ginger were significantly reduced the averaged fungus growth (*A. niger*) as it achieved a reduction of 6.16 and 6.31 cm respectively compared to 8.5cm in control group. The ginger concentrations of 5, 10 and 15 g L^{-1} were exceeded other concentrations and reduced the growth of the pathogenic fungus, so that it reached 5.15, 5.30 and 5.55 cm respectively, while 30 g L^{-1} recorded the lowest reduction on the fungus growth (6.58 cm) compared to 8.5 cm in control. The interaction results indicated that the 5 g L^{-1} cold ginger extract was exceeded other concentrations in the reduction of the pathogen growth as it achieved 5.10 cm, while, 30 g L^{-1} of hot extract recorded the lowest reduction on the fungus growth (6.70 cm) in comparison with 8.5 cm in control. The effect of ginger extract may attributed to the gingerol in ginger roots, since it is a mixture or crystals of gingerone that make the high acidity

of ginger which inhibit many microorganisms (Melvin *et al.* 2009; Rostamzad *et al.* 2019; Bayas-Morejón *et al.* 2020; Obaid *et al.* 2022). These results are in agreement with Hasan *et al.* (2005) who reported inhibition activity of ginger extract against *Aspergillus* sp. Tagoe *et al.* (2010) confirmed the effectiveness of plant extracts against *A. niger*, *A. flavus* and *Cladosporium*, as the ginger was among these plant extracts and showed a high inhibition activity against *A. niger*, *A. flavus* in particular, compared to other extracts.

Table 1. The effect of cold and hot aqueous extract of ginger on the growth of the pathogenic fungus *A. niger*.

The average of concentration	**Radial growth of *A. niger* (cm)**		**Ginger g L^{-1}**
	hot aqueous extract	**cold aqueous extract**	
5.15	5.20	5.10	5
5.30	5.40	5.20	10
5.55	5.70	5.40	15
6.18	6.20	6.15	20
6.43	6.50	6.35	25
6.58	6.70	6.45	30
8.50	8.50	8.50	control
	6.31	6.16	The average of factors
L.S.D 0.05	Concentration = 0.53	Factors = 1.12	Interaction = 0.66

*Each number in the table represents the average of three replicates.

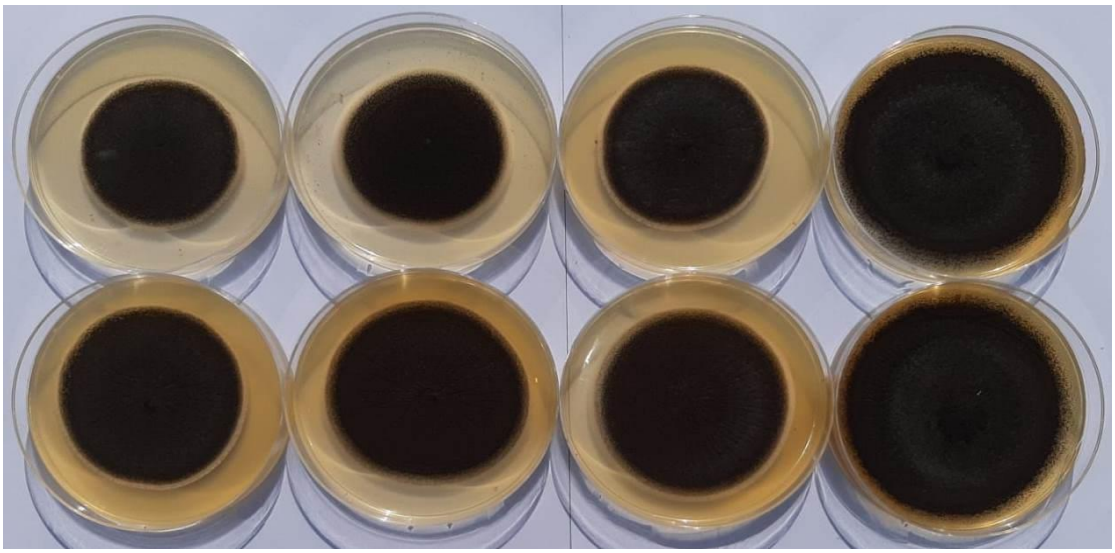

Fig. 1. The effect of cold aqueous extract of ginger on the growth of the pathogenic fungus *A. niger* (from the right: control, 30 g L^{-1}, 25 g L^{-1} and 20 g L^{-1}).

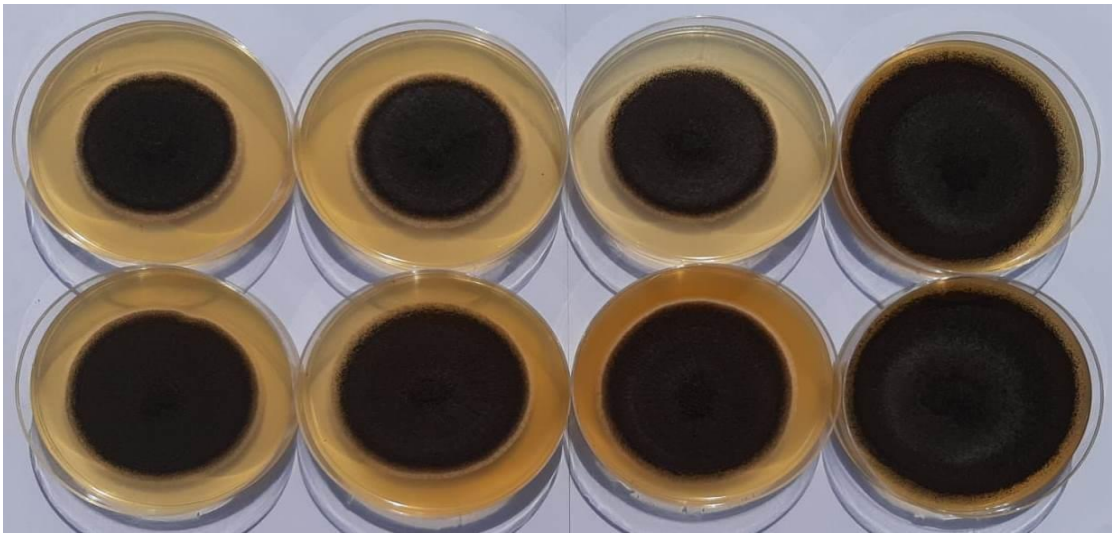

Fig. 2. The effect of hot aqueous extract of ginger on the growth of the pathogenic fungus *A. niger*. From the right (control, 30 g L^{-1}, 25 g L^{-1} and 20 g L^{-1}).

Table 2 showed the exceeding of spore suspension of *A. niger* + wounds treatment significantly compared to other treatments in increasing the amount of damage to the bulbs as it reached 7.63 cm, while, there was no damage occurred to the bulbs in wounds + ginger 5g L^{-1} treatment as it recorded 0 cm. The average of effect of weeks on the amount of damage to the bulbs indicated that the third week was the most affective week in the increasing the amount of damage to the bulbs as it reached 3.00 cm. The interaction showed that the spore suspension of *A. niger* + wounds and 30 g L^{-1} ginger increased the amount of damage to the bulbs up to 5.58 cm and 0.17 cm in *A. niger* + 5 g L^{-1} treatment. The reason for this may be attributed to the germination of fungus spores in wounds far from the effect of ginger extract. The positive effect of ginger extract may belong to active substances that inhibit the growth of the pathogenic fungus including zingberene, cucumene, (-)-β-sesquiphellandrene and β-basabolene (Duta 2005). In addition to the carbohydrates, flavonoid and alkaloid exhibit inhibition effect against many fungi (Chrubasik *et al.* 2005).

Table 2. Effects of dipping bulbs in cold aqueous extract of ginger on the infection by the pathogenic fungus *A. niger*.

Treatments	**The amount of damage cm/number of weeks**			**The average of treatments**
	First	**Second**	**Third**	
Distilled water only	0.00	0.00	0.00	0.00
Spore suspension of *A. niger*	4.77	5.13	8.00	6.30
Wounds only	1.66	2.83	3.36	2.62
Spore suspension of *A. niger* + wounds	6.88	8.00	8.00	7.63
Ginger 5 (g L^{-1})	0.00	0.00	0.00	0.00
Ginger (15 g L^{-1})	0.00	0.00	0.00	0.00
Ginger (30 g L^{-1})	0.00	0.00	0.00	0.00
Spore suspension of *A. niger* + Ginger (5 g L^{-1})	0.00	0.00	0.50	0.17
Spore suspension of *A. niger* + Ginger (15 g L^{-1})	0.00	0.65	1.15	0.60
Spore suspension of *A. niger* + Ginger (30 g L^{-1})	0.00	2.10	3.70	1.93
Wounds + Ginger (5 g L^{-1})	0.00	0.00	0.00	0.00
Wounds + Ginger (15 g L^{-1})	0.00	0.15	0.40	0.18
Wounds + Ginger (30 g L^{-1})	0.00	0.50	0.85	0.45
Spore suspension of *A. niger* + wounds + Ginger (5 g L^{-1})	1.63	2.16	6.12	3.97
Spore suspension of *A. niger* + wounds + Ginger (15 g L^{-1})	2.87	3.33	8.00	5.06
Spore suspension of *A. niger* + wounds + Ginger (30 g L^{-1})	3.13	4.60	8.00	5.58
The average of weeks	1.30	1.84	3.00	
L.S.D 0.05		Weeks = 0.29	treatments = 0.39	Interaction = 0.55

*Each number in the table represents the average of three replicates.

Results of Table 3 indicated the increasing in the amount of damage to the bulbs in spore suspension of *A. niger* + wounds treatment as it reached 7.63 cm, while it was 0.16 cm in the treatment containing wounds + 5 g L^{-1} Ginger. The effect of the number of weeks showed that the third was the most effective in the increasing the amount of damage to the bulbs when it recorded 3.37 cm. The interaction between spore suspension of *A. niger* + wounds and 30 g L^{-1} Ginger increased the amount of damage to the bulbs to 6.60 cm, while, the damage in spore suspension of *A. niger* + 5 g L^{-1} Ginger reached 0.53 cm. Ginger contains about 400 different compounds that affect fungi such as Gingerols, Shogaols, Zingerone and Sesquiterpenoids (Grzanna *et al.* 2005). Moreover, Alam *et al.* (2002) observed the effect of some plant extracts to inhibit conidiospores of some fungi including ginger. Table 3 indicated the low effect of ginger on the growth of *A. niger* particularly in the high concentrations which may be attributed to the fact that *A. niger* breaks compounds on the cell wall and prevents their entrance to the fungus cells to damage.

CONCLUSION

Results of current study indicated that the low concentrations of ginger extract were more effective to reduce the growth of the pathogenic fungus *A. niger*. The low effect of ginger on the growth of *A. niger* particularly in the high concentrations which may be attributed to the fact that the effectiveness of *A. niger* is to break compounds on the cell wall increased and prevent the entrance to the fungus cells and damage it.

Table 3. The effect of dipping bulbs in hot aqueous extract of ginger on the infection by the pathogenic fungus *A. niger*.

Treatments	The amount of damage cm/number of weeks			The average of treatments
	First	Second	Third	
Distilled water only	0.00	0.00	0.00	0.00
Spore suspension of *A. niger*	4.77	5.13	8.00	5.97
Wounds only	1.66	2.83	3.36	2.67
Spore suspension of *A. niger* + wounds	6.88	8.00	8.00	7.63
Ginger (5 g L^{-1})	0.00	0.00	0.00	0.00
Ginger (15 g L^{-1})	0.00	0.00	0.00	0.00
Ginger (30 g L^{-1})	0.00	0.00	0.00	0.00
Spore suspension of *A. niger* + Ginger (5 g L^{-1})	0.00	0.50	1.10	0.53
Spore suspension of *A. niger* + Ginger (15 g L^{-1})	0.00	1.85	2.35	1.40
Spore suspension of *A. niger* + Ginger (30 g L^{-1})	0.00	3.25	4.63	2.63
Wounds + Ginger (5 g L^{-1})	0.00	0.18	0.30	0.16
Wounds + Ginger (15 g L^{-1})	0.00	0.25	0.96	0.40
Wounds + Ginger (30 g L^{-1})	0.20	0.67	1.77	0.88
Spore suspension of *A. niger* + wounds + Ginger (5 g L^{-1})	2.86	3.44	7.39	4.56
Spore suspension of *A. niger* + wounds + Ginger (15 g L^{-1})	4.37	6.12	8.00	6.16
Spore suspension of *A. niger* + wounds + Ginger (30 g L^{-1})	4.66	7.16	8.00	6.60
The average of weeks	1.59	2.49	3.37	
L.S.D 0.05		Weeks = 0.37	treatments = 0.46	Interaction = 0.63

*Each number in the table represents the average of three replicates.

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