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In vitro Evaluation of Botanical Extracts against Some Soil and Seed Borne Fungi of Economic Importance

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Introduction: The investigation was done to observe the effectiveness of botanical extracts against *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii*. Biologically based and environmentally safe alternatives are needed for the management of pathogenic micro-organisms. Plant extracts might be a substantial alternative of chemical pesticides.
Aims: To investigate the effectiveness of three botanical extracts namely garlic, ginger and neem at different concentrations (5%, 10% and 15%) on the mycelial growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii.*
Study Design: *In vitro* testing.
Place and Duration of Study: The study was taken in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University from June 2011 to June 2012.
Methodology: Poison food technique was followed to measure the effect of botanical extracts. The inhibition percentages of the tested fungi were calculated based on the growth of the pathogen on PDA plates in absence of crude extracts by following the formula of Sunder et al. (1995). The plates were arranged in Completely Randomized Design (CRD) with five replications. The replicated plates were used for each treatment under each experiment and completely randomized

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design was followed for data analysis.
Results: The different botanical extracts in different concentrations inhibited the mycelial growth of fungi significantly ($p<0.01$). At 15% concentration garlic maximum inhibited *S. rolfsii* 72.20%, neem and ginger maximum inhibited in *F. oxysporum* 56.41% and 55.80% respectively.
Conclusion: The mycelial growth inhibition of soil and seed borne fungi was found to be increased with the increase of concentration of botanical extracts.

Keywords: Botanical extract; soil and seed borne fungi; poison food technique.

1. INTRODUCTION

Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratories trials [1,2]. Many plant and plant products have been reported to be antimicrobials against plant pathogenic fungi [3,4,5]. Ginger, garlic and neem found effective against *S. rolfsii* [6], *F. oxysporum* and *B. sorokiniana* [7,8]. The microbial inoculants as biocontrol agents are effective and attractive alternatives to prevent the deficiencies brought about by the exclusive reliance on chemicals [9]. Plant extracts might be a substantial alternative of chemical pesticides in controlling plant diseases.

Biologically based and environmentally safe alternatives are being investigated for possible use as components in integrated management programs. Therefore systematic research are needed to explore the potential of botanical extracts like neem, garlic, ginger etc against seed borne and soil borne fungal pathogens. Considering the above fact the present investigation was undertaken to investigate the effectiveness of selected botanical extracts against *F. oxysporum*, *B. sorokiniana* and *S. rolfsii.*

2. MATERIALS AND METHODS

Three isolates of *B. sorokiniana*, *F. oxysporum*, *S. rolfsii* were collected from the preserved isolates of Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, Khulna.

2.1 Preparation of Potato Dextrose Agar (PDA) Medium

PDA was prepared following the standard procedure [10]. 200 gm potato slice was boiled in 1000 ml distilled water. After that it was sieved and 20 gm dextrose was mixed with it. Then 15 gm agar was mixed with slowly and melted on hot plate magnetic stirrer. After preparation of PDA medium, it was poured in to 500 ml conical flasks then the conical flask was plugged with cotton plug and covered by brown paper. Finally the medium was sterilized in an autoclave at 121ºC temperature for 15 minutes.

2.2 Multiplication of *B. sorokiniana*, *F. oxysporum* and *S. rolfsii*

PDA was poured in sterilized petridishes, 20ml in each plate. After solidification, discs were cut with flame sterilized cork borer (5 mm diameter), then the plates were inoculated by placing 5 mm discs of PDA culture of isolated pathogens. The inoculated petridishes were kept in the growth chamber (25±2 ºC) for observation. All the works were undertaken under aseptic condition.

2.3 Preparation of Botanical Extracts

Different botanical extracts were evaluated in *in-vitro* condition against *B. sorokiniana*, *F. oxysporum* and *S. rolfsii* by following poison food technique [11].

2.4 Botanical Extracts and Their Concentration

Different botanical extracts which were used in this experiment and their concentration ratio are given in Table 1.

2.5 Preparation of Extract

2.5.1 Neem extract

Fresh leaves of neem were collected directly from neem trees. The leaves were washed with tap water and were taken into mortar and pestle. Then 5 ml, 10 ml and 15 ml pure neem extracts were mixed separately with 95 ml, 90 ml and 85 ml PDA respectively in separate sterilized 250 ml conical flasks to prepare 5%, 10% and 15% extract concentrations.

Table 1. List of plant materials that were used in the experiment

Common name	Botanical name	Family	Plant parts used	Concentration (%)
Neem	*Azadirachta indica*	Meliaceae	Leaves	5, 10 & 15
Garlic	*Allium sativum*	Alliaceae	Bulb	5, 10 & 15
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	5, 10 & 15

2.5.2 Garlic extract

Fresh bulbs of garlic were collected from the market. The bulbs were taken into mortar and pestle and pure extract was collected. Then 5 ml, l0 ml and 15 ml pure garlic extracts were mixed separately with 95 ml, 90 ml and 85 ml PDA respectively in separate 250 ml sterilized conical flask to prepare 5%, 10% and 15% extract concentrations.

2.5.3 Ginger extract

Fresh rhizomes of ginger were collected from the market. The rhizomes were washed and taken into mortar and pestle without mixing water and pure extract was collected. Then 5 ml, l0 ml and 15 ml pure ginger extracts were mixed separately with 95 ml, 90 ml and 85 ml PDA respectively in separate 250 ml sterilized conical flasks to prepare 5%, 10% and 15% extract concentrations.

2.6 *In vitro* Effect of Botanical Extracts on Mycelial Growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii*

An *in vitro* study was conducted to find out the effect of botanical extract against of *B. sorokiniana*, *F. oxysporum*, *S. rolfsii*. The basic PDA medium was modified by using the crude extracts at specified concentrations. The plates were inoculated with 5 mm discs from old PDA culture at one disc at centre per plate. Discs of mycelium (5 mm diameter) of tested fungi were cut from the edge of an actively growing fungal colony with a cork borer (5 mm diameter). The plates were incubated in growth chamber for 25±2℃.Thereafter inhibition percentages of the tested fungi were calculated based on the growth of the pathogen on PDA plates in absence of crude extracts following the formula suggested by [12].

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where

X = Average growth of tested fungi in control petridishes

Y = Average growth of tested fungi in each botanical extract treated petridishes

2.7 Experimental Design and Data Analysis

The plates were arranged in Completely Randomized Design (CRD) with five replications. The replicated plates were used for each treatment under each experiment and completely randomized design was followed for data analysis. The data were analyzed statistically using MSTAT-C computer program and means were compared for difference following Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Effect of Botanical Extracts on Mycelial Growth of *B. sorokiniana* at Different Concentration

The different botanical extracts in different concentrations inhibited the mycelial growth of *B. sorokiniana* significantly ($p<0.01$).

3.1.1 Garlic extract

The mycelial growth inhibition in different concentration of garlic bulb extracts was significantly increased with the increase of concentration (Table 2, Plate 1 and Fig. 1). The highest percent inhibition (31.14%) was observed at 15% concentration and the lowest percent inhibition (4.74%) was observed at 5% concentration. From regression equation (Fig. 1) between concentration of garlic extracts and mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. Extracts of garlic bulbs were found effective against *B. sorokiniana* by many scientists [7,8,13,14,15,16].

3.1.2 Neem extract

The mycelial growth inhibition in different concentration of neem leaves extracts was significantly increased with the increase of

concentration (Table 2, Plate 1 and Fig. 1). The highest percent inhibition (40.62%) was observed at 15% concentration and the lowest percent inhibition (24.33%) was observed at 5% concentration. From regression equation (Fig. 1) between concentration of neem extracts and mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. [16] were found neem extract as superior among the selected extracts followed by garlic, bishkatali and vatpata against *B. sorokiniana.* [7] examined ten plant extracts among them neem extract showed good inhibition against *B. sorokiniana*. [17] were found neem extracts to be effective against *B. sorokiniana*. [8] found that neem (*Azadirachta indica*) extracts were effective against seed borne infection *B. sorokiniana*. [15] also found the similar observation. [18] was found the effect of neem extracts in controlling root-knot nematode (*Meloidogyne javanica*). Present study also showed neem extracts was highly effective against *B. sorokiniana*.

3.1.3 Ginger extract

The mycelial growth inhibition in different concentration of ginger rhizome extracts was significantly increased with the increase of concentration (Table 2, Plate 1, and Fig. 1). The highest percent inhibition (38.35%) was observed at 15% concentration and the lowest percent inhibition (10.72%) was observed at 5% concentration. Regression equation (Fig. 1) between concentration of ginger extracts and mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. Similar observation was found by [7,8].

3.2 Effect of Botanical Extracts on Mycelial growth of *F. oxysporum* at Different Concentration

The different botanical extracts in different concentrations inhibited the mycelial growth of *F. oxysporum* significantly ($p<0.01$).

3.2.1 Garlic extract

The mycelial growth inhibition in different concentration of garlic bulb extracts was significantly increased with the increase of concentration (Table 3, Plate 2 and Fig. 2). The highest percent inhibition (48.72%) was observed at 15% concentration and the lowest percent inhibition (5.59%) was observed at 5% concentration. From regression equation (Fig. 2) between concentration of garlic extracts and mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. Present study also found similar with the [16,19,20,21].

Table 2. Effect of different botanical extracts at different concentration on mycelial growth inhibition of *B. sorokiniana*

Botanical extracts (%)	Concentration	Inhibition percentage
Control	**0**	**-**
Ginger	5	10.72ef
	10	24.12d
	15	38.35ab
Neem	5	24.33d
	10	33.40bc
	15	40.62a
Garlic	5	4.74f
	10	15.88e
	15	31.14c
CV (%)		12.62%
LSD value		6.643
Level of significance		0.01

Table 3. Effect of different botanical extracts at different concentration on mycelial growth inhibition of *F. oxysporum*

Botanical extracts (%)	Concentration	Inhibition percentage
Control	**0**	**-**
Ginger	5	9.49f
	10	31.28c
	15	55.80a
Neem	5	17.95e
	10	29.62cd
	15	56.41a
Garlic	5	5.59g
	10	27.82d
	15	48.72b
CV (%)		4.28%
LSD value		2.852
Level of significance		0.01

3.2.2 Neem extract

The mycelial growth inhibition in different concentration of neem leaves extracts was significantly increased with the increase of concentration (Table 3, Plate 2 and Fig. 2). The

highest percent inhibition (56.41%) was observed at 15% and the lowest percent inhibition (17.95%) was observed at 5%. Regression equation (Fig. 2) between concentration of neem extracts and mycelial growth inhibition percentage revealed that more than 95% of the variation in increased inhibition percentage could be explained by the increase of concentration. [16] were found neem extract as superior among the selected extracts followed by garlic, bishkatali and vatpata against *Fusarium spp.* Srivastava *et al* 2011 examined the same effect of neem leaf extract at different concentrations which was significant to manage the growth of *F. oxysporum*. Findings of [8,22] which were agree with that of present study.

3.2.3 Ginger extract

The mycelial growth inhibition in different concentrations of ginger rhizome extracts was significantly increased with the increase in concentration (Table 3, Plate 2, and Fig. 2). The highest percent inhibition (55.80%) was observed at 15% concentration and the lowest percent inhibition (9.49%) was observed at 5% concentration. Regression equation (Fig. 2) between concentration of ginger extracts and

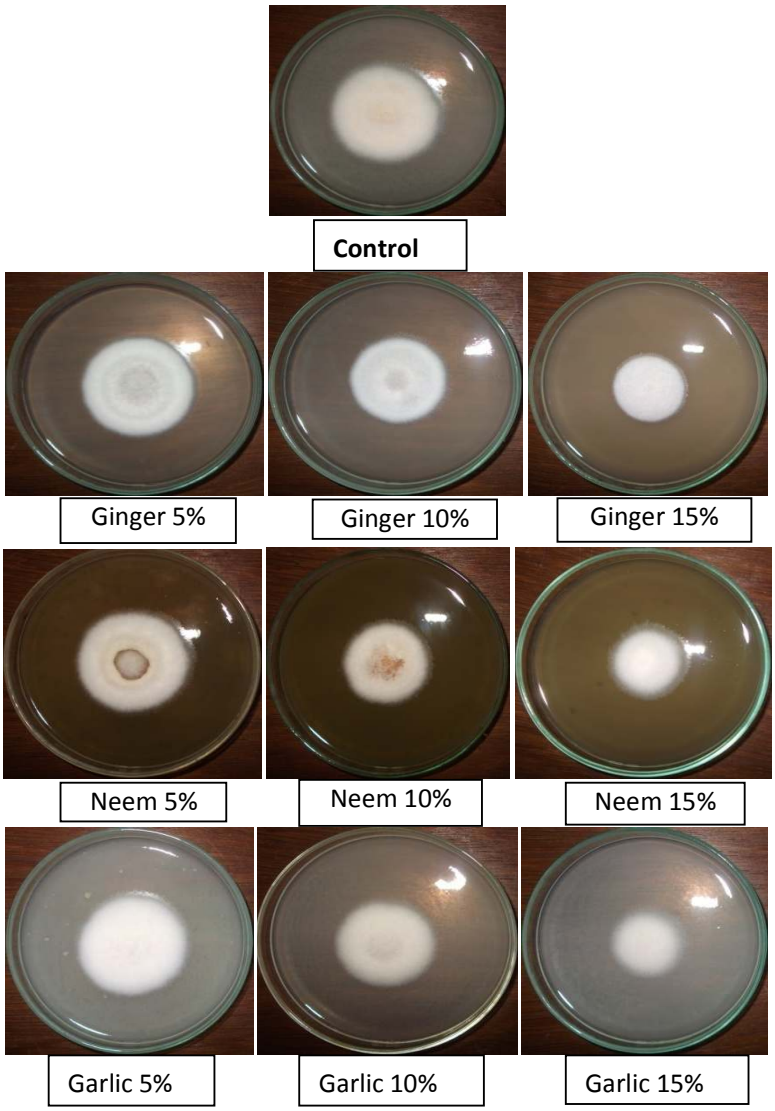

Plate 1. Effect of different botanical extracts at different concentration on mycelial growth of *B. sorokiniana*

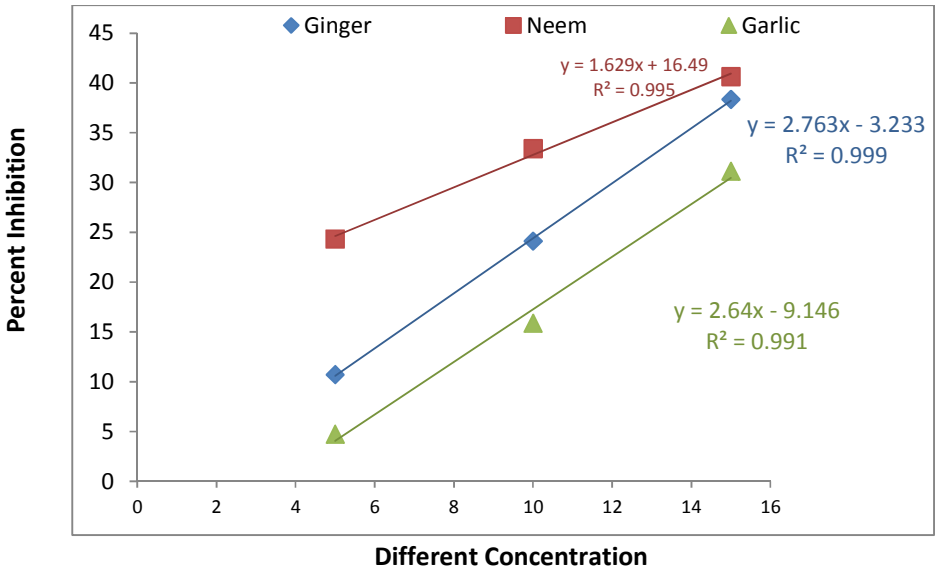

Fig. 1. Functional relationship between concentration and mycelial growth inhibition percentage of botanical extracts of *B. sorokiniana*

mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. Similar observation was found *by* [8,22,23] investigated that hot water extracts were obtained from Ginger (*Zingiber officinale*) was fungi toxic against *F. oxysporum* and the extract suppressed the growth of these fungi in culture.

3.3 Effect of Botanical Extracts on Mycelial Growth of *S. rolfsii* at Different Concentration

The different botanical extracts in different concentrations inhibited the mycelial growth of *S. rolfsii* significantly ($p<0.01$).

3.3.1 Garlic extract

The mycelial growth inhibition in different concentration of garlic bulb extracts was significantly increased with the increase of concentration (Table. 4, Plate 3 and Fig. 3). The highest percent inhibition (72.20%) was observed at 15% concentration and the lowest percent inhibition (32.35%) was observed at 5% concentration. From regression equation (Fig. 3) between concentration of garlic extracts and mycelial growth inhibition percentage revealed that more than 99% of the variation in increased inhibition percentage could be explained by the increase of concentration. [6] examined the same experiment at 5%, 10% and 20% (v/v) concentration where significantly highest 75.18% hyphal growth inhibition of *S. roflsii* was obtained with the highest concentration of garlic. [24] performed trials on the use of garlic bulb extract against *S. rolfsii* in the laboratory and in the nethouse which were highly promising. Present study also showed that garlic extracts was highly effective against *S. rolfsii.*

Table 4. Effect of different botanical extracts at different concentration on mycelial growth inhibition of *S. rolfsii*

Botanical extracts (%)	Concentration	Inhibition percentage
Control	0	-
Ginger	5	14.24f
	10	17.05e
	15	25.76d
Neem	5	4.17g
	10	12.28f
	15	26.14d
Garlic	5	32.35c
	10	53.94b
	15	72.20a
CV (%)		3.35%
LSD value		2.039
Level of significance		0.01

3.3.2 Neem extract

The mycelial growth inhibition in different concentration of neem leaves extracts was significantly increased with the increase of concentration (Table 4, Plate 3 and Fig. 3). The

highest percent inhibition (26.14%) was observed at 15% concentration and the lowest percent inhibition (4.17%) was observed at 5% concentration. From regression equation (Fig. 3) between concentration of neem extracts and mycelial growth inhibition percentage revealed that more than 97% of the variation in increased inhibition percentage could be explained by the increase of concentration. [6] examined the same experiment at 5%, 10% and 20% (v/v) concentration where significantly second highest 69.90% hyphal growth inhibition of *S. roflsii* was attained with the highest concentration of neem extract. [25] found that neem extracts were significantly reducing the viability of sclerotia.Present study also showed effectiveness of neem extracts against *S. rolfsii*.

3.3.3 Ginger extract

The mycelial growth inhibition in different concentration of ginger rhizome extracts was significantly increased with the increase of concentration (Table 4, Plate 3, and Fig. 3). The highest percent inhibition (25.76%) was observed at 15% concentration and the lowest percent inhibition (14.24%) was observed at 5% concentration. From regression equation (Fig. 3) between concentration of ginger extracts and mycelial growth inhibition percentage revealed that more than 91% of the variation in increased inhibition percentage could be explained by the increase of concentration. Similar observation was found by [6,26]

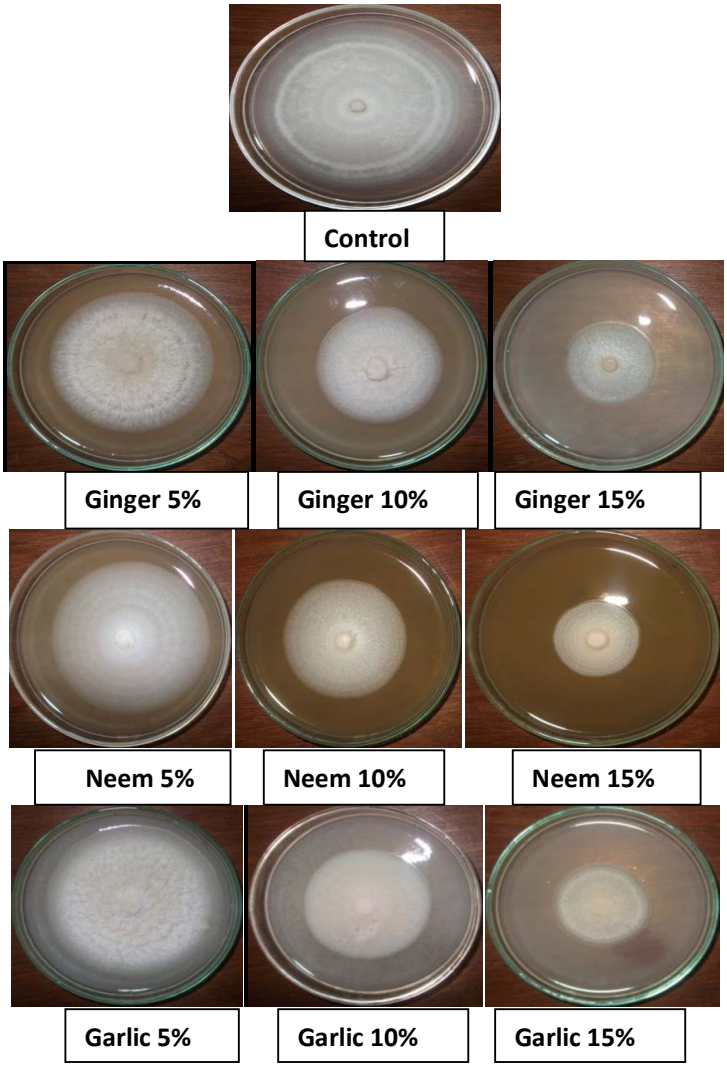

Plate 2. Effect of different botanical extracts at different concentration on mycelial growth of *F. oxysporum*

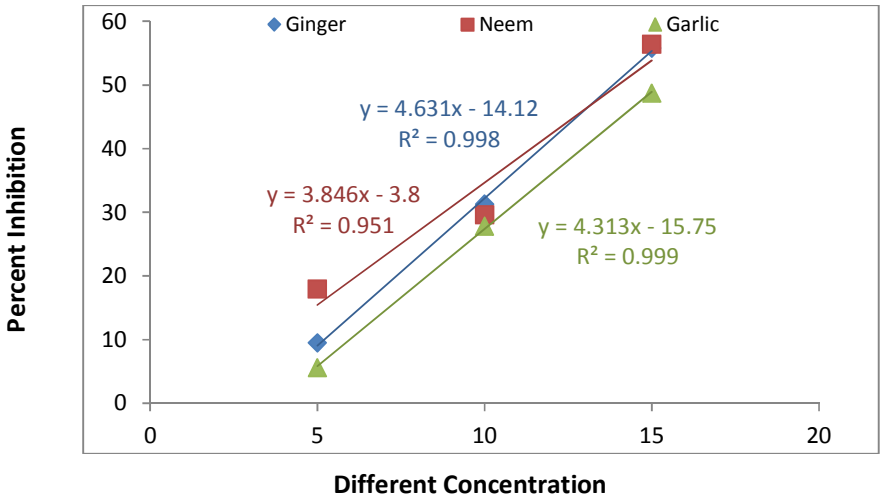

Fig. 2. Functional relationship between concentration and mycelial growth inhibition Percentage of botanical extracts of *F. oxysporum*

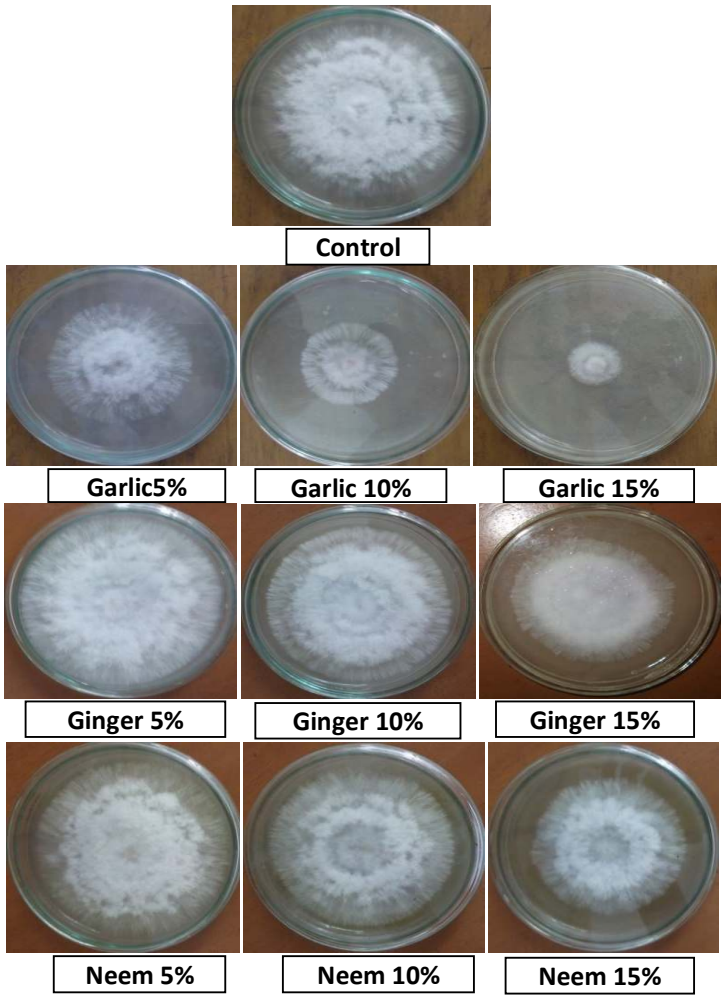

Plate 3. Effect of different botanical extracts at different concentration on mycelial growth of *S. rolfsii*

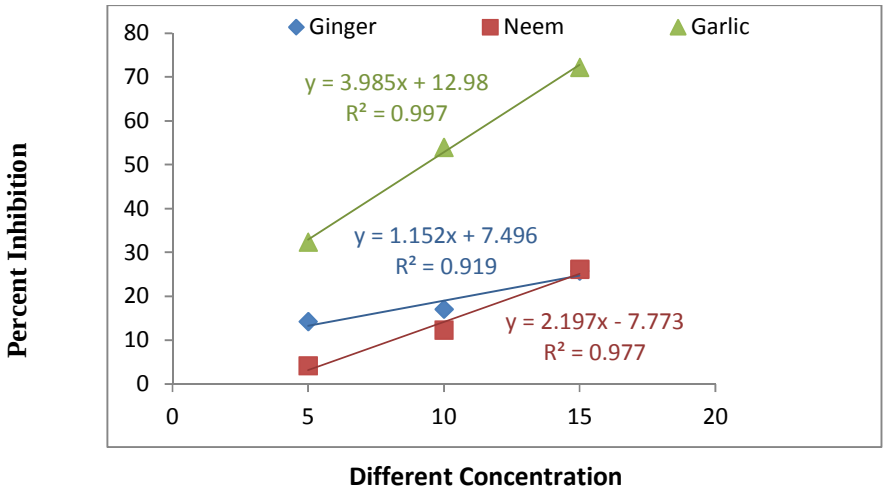

Fig. 3. Functional relationship between concentration and mycelial growth inhibition percentage of botanical extracts of *S. rolfsii*

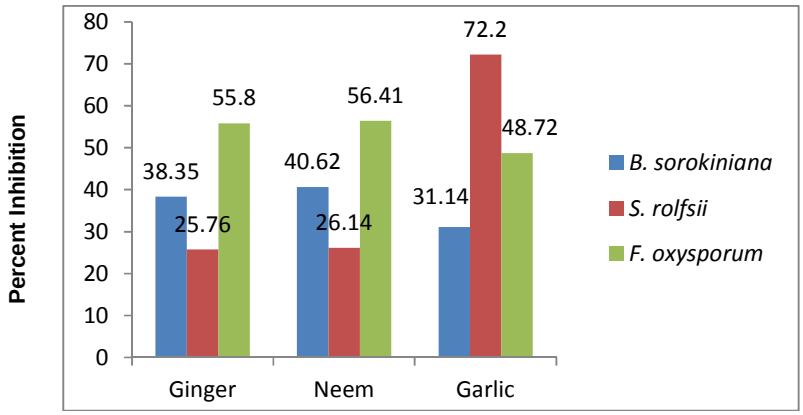

Fig. 4. Functional relationship between 15% concentration and mycelial growth percentage of botanical extracts of *F. oxysporum, B.sorokiniana, S. rolfsii*

3.4 Effect of Botanical Extracts on Mycelial Growth of *F. oxysporum, B. sorokiniana, S. rolfsii* at 15% Concentration

In neem and ginger extracts the highest percent inhibition of mycelial growth was observed in *F. oxysporum* that was 56.41% and 55.80% at 15% concentration respectively and in garlic extracts the highest percent inhibition of mycelial growth was observed in *S. rolfsii* that was 72.20% at 15% concentration. The mycelial growth inhibition was found to be increased with the increase of concentration (above Fig. 4).

4. CONCLUSION

Based on the evaluation of different botanical extracts, it can be concluded that mycelial growth inhibition percentages increased with the increasing in extracts concentration in all cases. The highest percent inhibition of mycelial growth of *Bipolaris sorokiniana* and *Fusarium oxysporum* observed by the application of neem extracts that was 40.62% and 56.41% at 15% concentration respectively and in *Sclerotium rolfsii* the highest percent inhibition of mycelial growth was observed by the application of garlic extracts (72.20%) at 15% concentration. Further investigation may be conducted with more treatments to confirm this result and generation of more information.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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