Antifungal properties of onion (*Allium cepa*), Ginger (*Zingiber officinale*) and Garlic (*Allium sativum*) on *Alternaria solani in vitro*

ABSTRACT

Plant pathogens cause serious losses in quantity and quality of agricultural products. Use of fungicides is gradually becoming unpopular due to their negative effects on ecosystems, human and animal health, and due to resistance by pathogens to the fungicides. In vitro studies were carried out in order to determine the effects of three plant extracts; onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) on the control of *Alternaria solani.* The experiment was laid in a Completely Randomized Design (CRD) with a 3*3 factorial arrangement plus one control. The first factor was plant extract, with three levels (garlic, onion and ginger) the second was plant extract concentration, with three levels (50%, 75% and 100%). The experiment was carried out in the laboratory at Midlands State University, Zimbabwe, in October 2014.Data on mycelia growth diameter, mycelia inhibition percent and spore germination percent was collected. Analysis of variance was done using Genstat 14th edition and means were separated using Duncan Multiple Range Test at 5% level of significance. Results showed that the plant extracts had strong antifungal activity and their effect increased with increase in their concentration. Ginger and garlic had significantly stronger effect on reducing mycelia growth, reducing spore germination and causing high inhibition per cent of *A. solani.* Ginger was the most effective in controlling *A. solani* across all concentrations. It can be concluded that the plant extracts (onion, ginger and garlic) can be used as natural fungicides to control pathogenic fungi. It is recommended that further research be done on the plant extracts so as to identify the active compounds which are in the extracts as these are responsible for this fungicidal activity and to carry out more studies to test antifungal activities of these studied plant extracts on other different fungi, at different concentration levels. Further experiments may also be done in the field to determine effects of these plant extracts in controlling diseases caused by *A. solani*.

Key words: Antifungal activity, plant extracts, in vitro, *Alternaria solani,* concentrations

1.0 INTRODUCTION

In agriculture, the crop loss due to plant pathogens has become a major concern and one of such pathogens is *Alternaria solani*. Alternaria solani is a soil inhabiting air borne pathogen [1] responsible for early blight, an important chronic foliar disease of mainly the Solanacea family including tomatoes (*Lycopersicon esculentum*) and potato (*Solanum tuberosum*) [2]. Basal girdling and death of seedlings may occur, a symptom known as collar rot. Despite the name "early," foliar symptoms usually occur on older leaves, [3]. The disease causes yield losses through defoliation of plants and this may result in a reduction in yields by as much as 20 to 30% for example in potatoes [4].

Chemical control is the most effective and applied method in controlling *A.solani* and there are numerous fungicides on the market for controlling early blight. The disease is commonly managed using succinate dehydrogenase inhibitor (SDHI) fungicides. Unfortunately, recent studies have shown that SDHI resistance has increased dramatically over the years in *A. solani* populations [5]. In addition, conventional pesticides; over the past five decades have led to a range of problems in agriculture, the environment, and human health [6]. There are numerous costs derived from pesticide use and these include monitoring and sanitation for contamination of soils, drinking water, or food, poisoning of pesticide users and farm workers, and the deleterious effects on non-target organisms such as bees and other beneficial insects, fish, and birds [7]. To overcome these problems, some alternative control methods must be employed.

Natural plant products (botanicals) are becoming a new source of agricultural chemicals to manage plant diseases [8]. Plant extracts have been known for their medicinal and antimicrobial properties since ancient times [9]. Many higher plants produce economically important organic compounds, pharmaceuticals and pesticides. Plant based secondary metabolites, which have defensive role may be exploited for the management of foliar diseases, [10]. The antifungal action of plant extracts has gained much attention. Nowadays, plants are being used against many plant pathogenic fungi. The plants serve as eco-friendly and economic bio-control agents [11]. Natural chemicals from plants are cheap, readily available and cost-effective in developing countries where synthetic fungicides are scarce and expensive for resource-poor farmers, [12]. A number of researches have been documented which demonstrate the antimicrobial efficacy of various plant extracts which have been seen to contain some antifungal properties against *Alternaria solani*. These botanicals include onions, (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum* [11]; [13]; [14]. These three botanicals have antifungal properties, which enable them to distort the life cycle of *A.solani* [15]. The present study was designed to evaluate the efficacy of three plant extracts, onion, ginger and garlic on *Alternaria solani* in vitro.

2.0 MATERIALS AND METHODS

2.1 Site description and experimental design

The experiment was carried out in the laboratory at Midlands State University which is located in Gweru, Zimbabwe. The area is found in Agro-ecological Region III [16] on the following coordinates 29°45'E, 19°45'S and the altitude is 1420m above sea level.

The experiment was laid in a Completely Randomized Design (CRD) with a 3*3 factorial arrangement plus one control. The first factor was plant extract type, with three levels; garlic, onion and ginger, while the second factor was plant extract concentration, with three levels; 50%, 75% and 100%.The control used was ethanol. The experiment was replicated three times.

2.2 Experimental Procedure

2.2.1 Isolation of *A. solani*.

Tomato leaves showing symptoms of the disease were collected from naturally infected tomato plants in a greenhouse. Initial symptoms on leaves appear as small 1-2 mm black or brown lesions and under conducive environmental conditions the lesions will enlarge and are often surrounded by a yellow halo. Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. As lesions expand and new lesions develop entire leaves may turn chlorotic and dehisce, leading to significant defoliation [3]. The infected tissues along with adjacent small unaffected tissue are cut into small pieces (2–5 mm squares) and by using flame-sterilized forceps, they are transferred to sterile Petri dishes containing 5% sodium hypo chloride for 30-60 s for surface sterilization of plant tissues. The sterilized pieces are aseptically transferred to Petri dishes containing solidified PDA and were incubated at 27^{0}C for 72 hours as according to [17].

2.2.2 Preparation of plant extracts and inoculation of *A.solani*

The research material ginger (*Z. officinale*) rhizomes, onion (*A. cepa*) bulbs, and garlic (*A. sativum)* bulbs, was obtained from a local vegetable market. Fifty grams of the plant material of each plant species was washed with water and crushed in a mortar with pestle by adding sterile distilled water at the rate of 10 ml/10g of plant tissue and the homogenates were centrifuged at 10 000 rpm for 15 min at 4℃ and the supernatant solutions were collected [18]. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120^{o}C for 30 min. The obtained extracts served as the crude extract which is the 100% concentration as according to [19]. The obtained concentrates were stored at 4℃. Out of the 100% crude extract from the different plant materials, the respective dilutions of 50% and 75% were then prepared.

2.3 Determination of mycelia growth diameter

5ml of 50%, 75 % and 100% of natural concentrate of onion (*A. cepa*), garlic (*A. sativum*) and ginger (*Z. officinale),* was then administered separately into Petri dishes and blended with cooled liquid PDA. 1ml of ethanol (positive control) was poured per Petri dish using an inoculating needle. 15 ml PDA was separately poured into Petri dishes, allowed to cool and solidify. After complete solidification of the medium, 5mm disc of 72 hour old culture of the *A. solani* was inoculated into PDA at the centre of the Petri dishes. The plates were incubated at 28^o C. The Petri dishes containing media devoid of the extract but with same amount of distilled water served as control. *A. solani* mycelia growth diameter was measured using a string diagonally and the string was put on a 30cm measuring ruler. This was done daily for four consecutive days. Mean diameter was calculated respectively to plant type and concentration level.

2.4 Determination of mycelial Inhibition percentage by Poisoned food technique

After incubation the colony diameter was measured in mm [20]. Each treatment was repeated three times. The toxicity of the extracts in terms of percentage inhibition of mycelia growth was calculated using the formula: Gc - Gt/Gc * 100, where Gc =diameter in control and Gt= diameter in plant extract.

2.5 Spore germination

The counting of conidia was done by means of haemocytometer for this purpose one disc (1 cm) from each Petri dish was taken from 7 days old culture of *A. solani*. The disc (1 cm) was washed using 2ml of distilled water for the collection of spores. One drop of solution was put on haemocytometer and spores were counted under microscope. The percentage was found using the formula:

Number of spore germinated/number of examined spores *100

2.6 DATA ANALYSIS

Analysis of variance (ANOVA) was done on data collected using Genstat 14th edition. Separation of means was done using Duncan Multiple Range Test at 5% level of significance.

3.0 RESULTS

3.1 Effects of plant extracts on *A. solani* mycelia growth diameter

There was an interaction between plant type and concentration level of the plant extracts on mycelia growth diameter of *A. solani.* The mycelia colony diameter decreased with an increase in concentration rate of the different plant extracts. Of the three plant extracts, the highest mycelial growth diameter (3.7cm) was recorded for garlic at 50% concentration level while the lowest was recorded for ginger at 100% and this was not significantly different ($P<0.05$) from that of the control (ethanol). Generally ginger resulted in the highest decrease in *A. solani* colony diameter across all respective concentrations (50%, 75% and 100%) though its effect at 50% and 75% were not significantly different from that of onion at these respective concentrations (Fig 1).

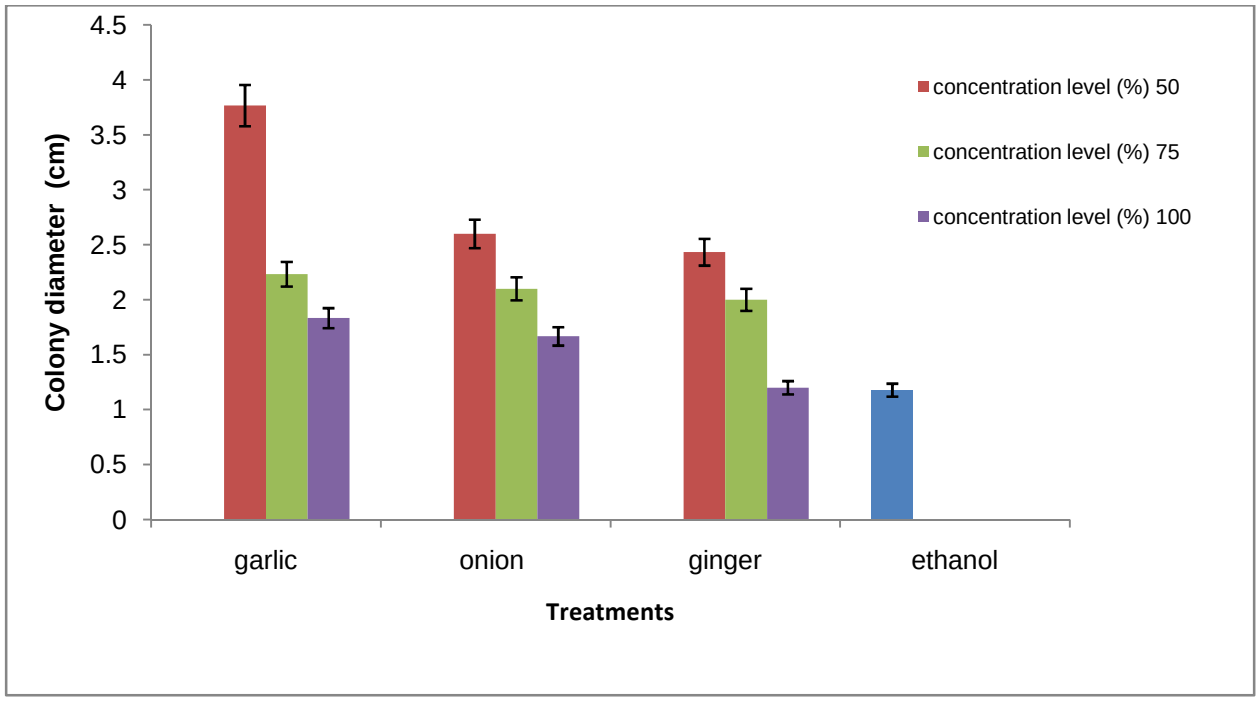

Fig. 1. Effects of plant extracts and different concentrations on mycelial diameter growth of *A.solani*

3.2 Effects of plant extracts on inhibition percentage

There was an interaction between plant type and concentration level on their effects on inhibition percentage. As the concentration of the plant extracts increased; the *A. solani* inhibition percentage also increased (Fig 1). Of the three plant extracts, garlic applied at 100% concentration resulted in the highest inhibition percentage followed by 100% onion although this was not significantly different ($P<0.05$) from that of 100% ginger. Ethanol (control) recorded the highest *A. solani* inhibition percentage (100%).

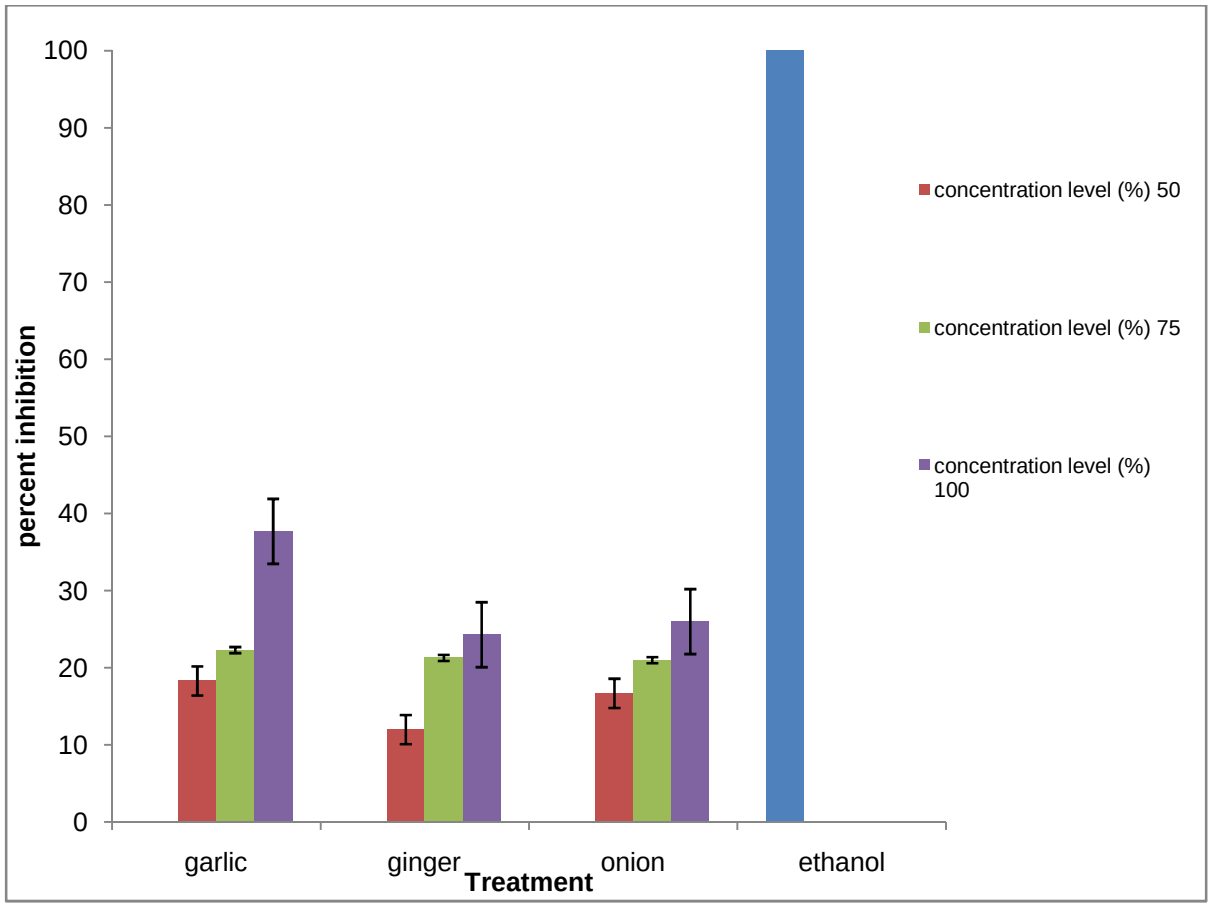

Fig. 2. Effects of plant extracts and concentrations on inhibition percentage of *A.solani*

3.3 Effects of plant extracts on spore germination

There was an interaction between plant extract type and concentration level on *A. solani* spore germination percentage. There was a reduction in spore germination percentage as concentration of the respective plant extracts increased. Results showed that ginger resulted in a significantly ($P<0.05$) greatest reduction in spore germination percentage while onion resulted in the highest spore germination percentage under the three concentration levels (Fig 3). Where ethanol (control) was used, no spores germinated at all.

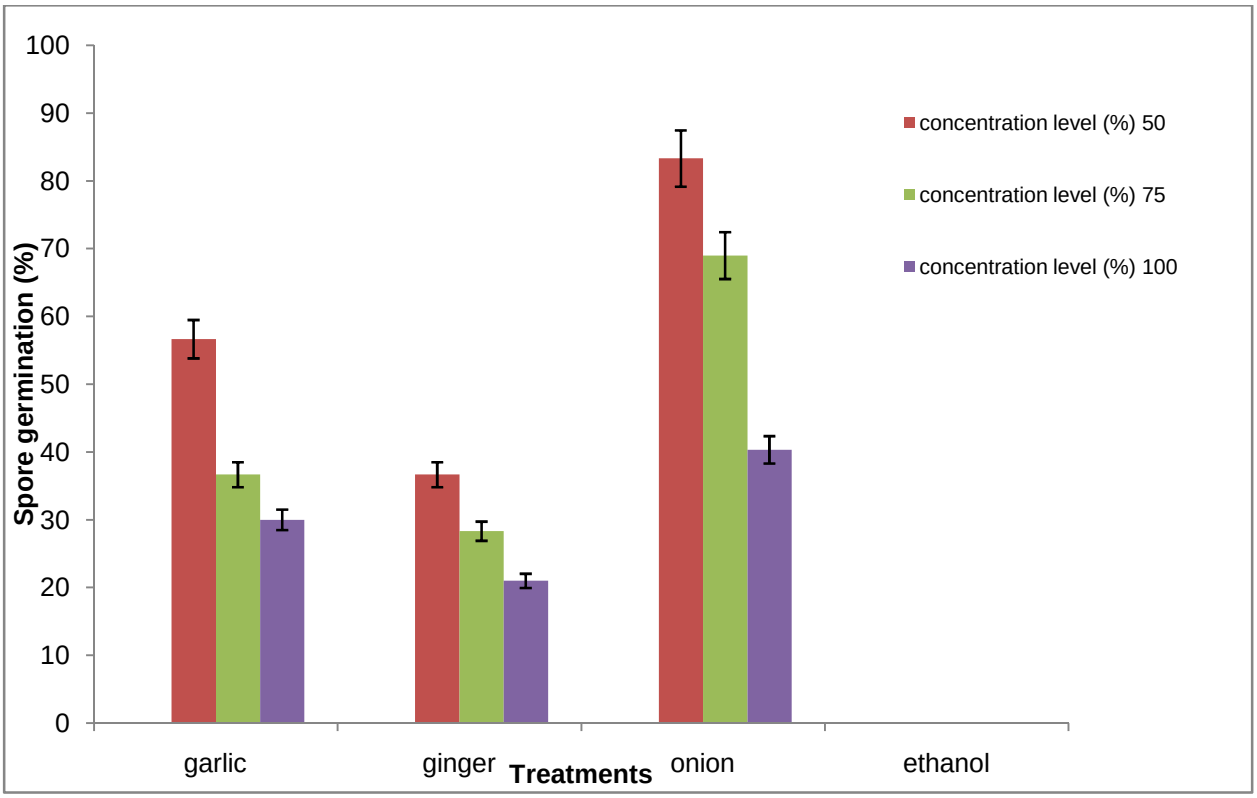

Fig. 3. Effects of plant extracts and concentrations on spore germination percent of *A.solani* .

4.0 DISCUSSION

The results from our study showed that the plant extracts tested (ginger, garlic and onion) have some antifungal properties and have the capacity to suppress development of *A. solani*. The reduction in mycelia growth increased with increase in concentration of the extracts. This is in concurrence with some in-vitro action tests conveyed on some plant extracts on seed borne pathogens of wheat, for example, *Aspergillus* spp [21]. Similar findings were reported by Swame and Alane, 2013 who found that at higher concentrations tested, plant extracts were effective in controlling seed borne fungi of mungbean seed. Tagoe *et al*, 2009 also noted the antifungal properties of garlic in inhibiting the growth of *Aspergillus* species. Results of this study are also in line with those of other researchers who showed that plant extracts result in inhibition of mycelial growth and these extracts include *Allium cepa* and *Allium sativum* [23], *Azadirachta indica* [13], *Zinger officinale* [14].

Ginger had the highest antifungal activity on *A. solani* with mycelial diameter mean of (2.4cm) at 50%, (2.1cm) at 75% and (1.2cm) at 100%. The strong inhibition potential of ginger is attributed to the fact that it contains over 400 different compounds, a mixture of both volatile and non-volatile chemical constituents such as zingerone, shogaols and gingerols, sesquiterpenoids (β-sesquiphellandrene, bisabolene and farnesene) and a small monoterpenoid fraction (β-phelladrene, cineol, and citral [24].The main constituents of the garlic essential oils are diallyl monosulfide, diallyl disulfide (DADS), diallyl trisulfide, and diallyl tetrasulfide [25]. G*ingerols* and *shogals*, found in ginger are less volatile as compared to *alliin* in garlic and onion which could have been lost through diffusion during plant extracts preparation process.

There was an interaction between plant extract type and concentration level on spore germination percentage. As plant extract concentration level increased, this resulted in a corresponding decrease in spore germination percentage. Ginger at 100 % was most effective with the lowest spore germination percentage of 22%. Results on the effectiveness of ginger as a bio control is in line with findings by Fawzi *et al*., 2009, who showed that plant extracts including cinnamon (*Cinnamomum zeylanicum*), laurel (*Laurus nobilis*) and ginger (*Zinger officinale*) had strong antifungal activity with high inhibition on growth of *Alternaria alternata* and *Fusarium oxysporum*. According to this study by Fawzi *et al*., 2009 ginger proved to be the most effective in inhibiting fungal growth, similar to our findings. Of the three extracts used garlic and ginger were comparatively most effective in controlling *A. solani*. This is in line with studies by Islam and Faruq, 2013, who also showed that garlic clove and ginger rhizome were effective in controlling *Fusarium oxysporum* and *Scleretonium rolfsii*; fungi which cause damping off disease. However on spore germination garlic across all concentrations turned to be more effective as compared to onion. This is likely because garlic is known to have some added phytochemicals which inhibit spore germination, [22]. These findings are in agreement with those of [27]; [28]; [29] who found the positive antifungal spore germination effect of the plant extracts *A. cepa* and *A. sativum.* Garlic has also been shown to effectively reduce mycelia growth of *Pythium aphanidermatum*, a causal organism of damping of chilli [30].

Experiment by Mohana and Raveesha 2007, confirmed the antimicrobial activity of six plant extracts including sweat Basil, neem, eucalyptus, Jimson weed, oleander and garlic, against *Alternaria solani* in vitro. In the study, neem and garlic were shown to be the most effective in causing highest reduction of mycelia growth of *A. solani* (43.3% and 42.2% respectively). The inhibitory effects of plant extracts may be due to their direct toxic effects on the pathogen or the plant extracts may induce systemic resistance in host plants resulting in a reduction of the disease development [31].

5.0 CONCLUSION AND RECOMMENDATIONS

From our findings it can be concluded that plant extracts onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) can be used for biocontrol of *A. solani* since they have antifungal properties. It has been demonstrated that these plant extracts extracts can effectively reduce *A. solani* mycelia growth, and cause significant inhibition of fungal growth. Of the plant extracts used; ginger proved to be most effective followed by garlic then onion. It can also be concluded that plant extracts may be more effective in fungal growth control at high concentrations. Use of plant extracts as control method of *A. solani* can contribute to minimizing risks and hazards of toxic fungicides. We recommend for further research to be done on the plant extracts so as to identify the active compounds which are in the extracts as these are responsible for this fungicidal activity. In addition, it is recommended that more studies be done to test antifungal activities of the studied plant extracts on other different fungi, at different concentration levels. Further experiments may also be done in the field to determine effects of these plant extracts in controlling diseases caused by *A. solani* for example early blight.

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