

Crude Garlic Extract Effect on the Growth of Mycelia, Germination of Zoospores and Sporangia and Time of Application on the Infection of *Phytophthora infestans* (Mont.) de Bary of Potato under Controlled Conditions in Ethiopia

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ABSTRACT

The experiments were carried out at Holetta, Ethiopia, Agricultural Research Center laboratory and green house conditions in 2004 crop season. The effects of 0.5 %, 2 %, 4 %, 6 % and 8 % concentrations of crude garlic extract and 0.1% fungicide Dithane M 45 (as positive control) and 0 % concentration (as negative control) on the inhibition of the growth of mycelia, germination of zoospore and sporangia of *Phytophthora infestans* were studied. In addition, the effect of time of applications of 2 % garlic extract on pathogen infection was tested on potato variety Awash in the greenhouse. Results of these studies provided evidence that all concentrations significantly ($p < 0.05$) inhibited the growth of the mycelia compared to the control and the tested concentrations showed highly significant difference ($p < 0.001$) on percent inhibition and germination of zoospores and sporangia compared to the negative control treatment. The 2 % garlic crude extracts showed curative effect on the development of *P. infestans* when it was applied at a time and one day before inoculation.

Key words: garlic extracts, *Phytophthora infestans*, mycelial growth, germination, zoospores, sporangia, infection

INTRODUCTION

Potato late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most economical diseases in the world (CIP, 1989) and in Ethiopia (Bekele, 1990). Bekele and Yaynu (1996) reported that, the disease attributed to an average tuber yield losses of 42 %. The main factors to high yield tuber losses are: 1) varieties in use are highly susceptible to this disease 2) poor;

degenerated seed tubers 3) poor crop management and low input. Though few farmers experiencing application of fungicides to control the disease, there is a knowledge gap in time of application and calibration thus, the effect is not reliable (Bekele and Mela, 2002). In addition to these, there are many alarming reports indicating the emergence of new fungicide resistant races belonging to various fungi groups including *P. infestans* (Davidse *et al.*, 1983; Schiessendoppler

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et al., 2003). However, the use of antifungal substances already present in these diet menus may provide a means to overcome these drawbacks attributed by the use of chemical fungicides.

Botanical pesticides have received great attention in recent years as an alternative to chemical pesticides (Wang *et al.*, 2004) and medicinal plants have shown much potential in controlling plant disease (Cao *et al.*, 2001). Wang *et al.* 2001, reported that, out of 88 species tested as an extract against *P. infestans*, extracts of 31 plant spp. completely inhibited the germination of sporangia, and extracts from 19 plant spp. inhibited to mycelium growth and sporangia germination (Wang *et al.*, 2001).

In search for such naturally occurring antifungal substances, many investigations have shown that garlic (*Allium sativum L.*) contains an active substance against bacteria and fungi as early as 1951 (Chaffer *et al.*, 1951). Mekuria *et al.* (1999) demonstrated the inhibition effect of biopesticides on *Phytophthora infestans* on tomato and the antifungal property of garlic extensively had been studied in the controlled environments (Sharma *et al.*, 1977). Extracts from garlic and other crops with volatile oils (e.g. Caraway) placed on the corner of the storage facilities inhibit sprouting and rotting of potato tubers (Cizcova *et al.*, 2002). The effectiveness of the substance from crude garlic extract on late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary was not studied much as suppose to be. However, some preliminary result (Sharma *et al.*, 1977 and Rahman and Motoyama, 2000) reports indicated the potential of crude garlic extract in inhibiting effect of the growth of the fungus.

It was thus felt of interest to examine the inhibition effect of different concentrations of crude garlic extract on the fungal growth, on zoospores and sporangia germination. In addition, the influence of time of application on infection of the *P. infestans* in vitro and in the green house respectively as the bases to develop confidence

for field studies was investigated.

MATERIALS AND METHODS

1. Preparation of the fungus: The laboratory manual developed by International Potato Center (CIP, 1997) was used to prepare the fungus. Sporulating young lesions in the leaf tissue were brought from potato fields to the laboratory. The fresh leaves with lesions were washed by distilled water and placed in petri dish with water agar with the leaf's axial side up. Plates were incubated at 15-18°C until sporulation appears (for a day) in humid chamber. Small pieces of infected tissue from the sporulating border of the lesions were cut and plated under potato slices in an empty Petri dish. Dishes were incubated at 15-18°C for a week, until there was abundant sporulation on the upper side of the slice. In order to harvest the inoculum, the fruiting body of the fungus were washed from the upper sides of sporulating potato slice with distilled water and passed through a 30 micron mesh filter which traps the sporangia. After washing several times with distilled water, the fruiting bodies were rubbed and collected from the filter with a small amount of sterile water. The fungus was kept on 15 % un-clarified V- 8 juice agar (CIP, 1997) Petri dishes under 16°C in darkness for sporulation. Cultures of 2-3 weeks old were used for all studies.

2. Preparation of Bulb Extract: Bulbs of fresh garlic were purchased from the local markets. Since there was no differences on its effect between dry and fresh bulb extract (Dixit, 1992), crude extracts of garlic from fresh bulb was used for all experiments. The bulbs were pilled and cut in to small pieces and 250 g from the lot was kept for an hour at room temperature and macerated and soaked in 1 l of 95 % ethyl alcohol for 48 hrs at room temperature. The soaked garlic was filtered using vacuum filter twice with Whiteman filters and distilled. The aqueous extract of garlic

(oil) was kept in the refrigerator at a temperature of mines 4 ± 1 as stock solution for all experiments (Nene *et al.*, 1968).

1.1 Effect of crude garlic extract on the growth of *P. infestans*.

An 8 mm diameter agar disk from a three-week *P. infestans* culture was transferred to the centers of 9 cm petri dish containing 15 ml of unclarified V8 – agar which has been previously amended with 0.5, 2, 4, 6 and 8% of garlic extract and 0.1% Dithane M 45 (Mancozeb 80 % WP) as positive control and water as negative control using 8 mm size cork borer and placed with a fungal side upward in the center of media.

The experimental was designed in CRD with 5 replications and was kept in the incubator at 15 -18°C in the dark with the surface downward. After one week the radial growth of the colony in each treatment was measured along two diameters at the right angle every day until the growth of the fungus in the control plot reached maximum (the time when it covered the circumference of the 9 cm petri dish). Fungi toxicity was expressed in terms of percentage of mycelial growth inhibition and calculated according to the formula $(dc - dt) / dc \times 100$, where dc = average diameter of fungal colony with control and dt = average diameter of fungal colony with treatment (Pander *et al.*, 1982).

1.2 Effect of crude garlic extract on the germination of *P. infestans*.

Garlic extract from the stock solution was taken and tested with different concentrations. Accordingly, crude garlic extract that had the concentrations of 0.5, 2, 4, 6, 8, and 0% as a negative control and 0.1 % Dithane M 45 (Mancozeb 80% WP) as a positive control were set immediately after the solution was prepared. In order to prepare the suspension of the sporangia and zoospore, 5 ml distilled water was added in the 15 days old *P. infestans* culture and the fruiting body was rubbed with glass rod and filtered The

collected sporangial suspension was incubated at 9°C to promote zoospore release. In order to separate sporangia with a size of 36×22 to $29 \times 19 \mu\text{m}$ from zoospores with an average size of $17 \times 16 \mu\text{m}$ (Erwin and Ribeiro, 1996), the suspension again passed through 10 micron mesh filter and the zoospores were separated from sporangia. Then the zoospores and the sporangia were separately subjected to germination test. The concentrations of the suspensions were calibrated to 10^5 ml measured by Hemacytometer (Hausser Scientific, Hosham PA, USA). The effect of the extract was studied by slide germination method as recommended by the American Phytopathological Society (Erwin and Ribeiro, 1996). The slides were kept in the plastic box with wet paper underneath and the box were put in the refrigerator at 4°C for 20 minutes to promote germination and then moved to into incubator (General Electric Press ion Scientific, PS) for 4h at 16°C. The numbers of germinated sporangia and zoospores were counted under the microscope and converted to percentage.

1.3 Effect of timing of application of crude garlic extract on the infection of *P. infestans*.

Tubers were taken from one of late blight susceptible potato (*Solanum tuberosum* L.) cultivar, awash. The tubers were produced in the screen houses being considered as sources of disease free seed stock produced through stem cuttings. The experimental design was CRD in 4 replications. Two tubers were planted in each pot and after a month they were thinned to maintain one plant per pot. After 45 days of growth, the plants were exposed to treatments. Spays were made with 2 % concentration of garlic extract, Dithane M 45 (Mancozeb 80 % WP) 0.1 % as a positive control and water as a negative control were used in the test. Sprays were made at 3, 2 and 1 day pre inoculation and 1, 2, 3 day post-inoculation and at the same time of inoculation. The whole plant were inoculated uniformly by

using a sprayer (ACE all purpose sprayer), which contained 500 ml of spore suspension per milliliter.

The number of lesions (L) on the plant was counted. The inhibition effect was determined relatively to the control plants and calculated by the following equation: % of inhibition = (No. of L (control) – No. of L (treatment) / No. of L (control)) × 100. In addition to lesion number lesion size were also measured.

RESULTS

Effect of crude garlic extract on the growth of *P. infestans*

Significant ($P < 0.05$) inhibition effect on the growth of the fungus of *P. infestans* was obtained using all rate of concentrations. The results in Table 1 indicated that the highest concentration 8 % crude garlic extract showed significantly highest (99.5 %) mycelia growth inhibition which was comparable to the 0.1 % fungicide amended treatment (100%). The difference in the growth of mycelia between 4 % and 6 % garlic extract concentrations did not vary significantly ($p < 0.05$) (Table 1). The inhibition effect declined as the concentrations reduced from 8 % to 0.05 %. The lowest inhibition effect was obtained with the lowest concentrations of the crude garlic extract (0.5 %) that was 16 % whereas

2 %, 4 % and 6 % concentrations had 31 %, 41 % and 40 % inhibition effects respectively. The growth of the mycelia was terminated 13 days after inoculation (Figure 1 and Figure 2) and this was the time where the growth of the fungus reached maximum in the water control in 9cm width petri dish.

Effect of crude garlic extract on the germination of *P. infestans*.

Data on the effect of crude garlic extract on the germination and inhibition of zoospores and sporangia are indicated in Table 2. The results obtained showed highly significant differences ($p < 0.001$) among the concentrations in respect to inhibition of the germination of zoospore and sporangia and had 100 % effect (Table 2). However a slight reduction of percent germination of sporangia (80 %) was recorded when 0.05 % garlic extract concentration was used. The remaining concentrations gave 100 % and 100% germination and inhibition effects on zoospores and sporangia respectively.

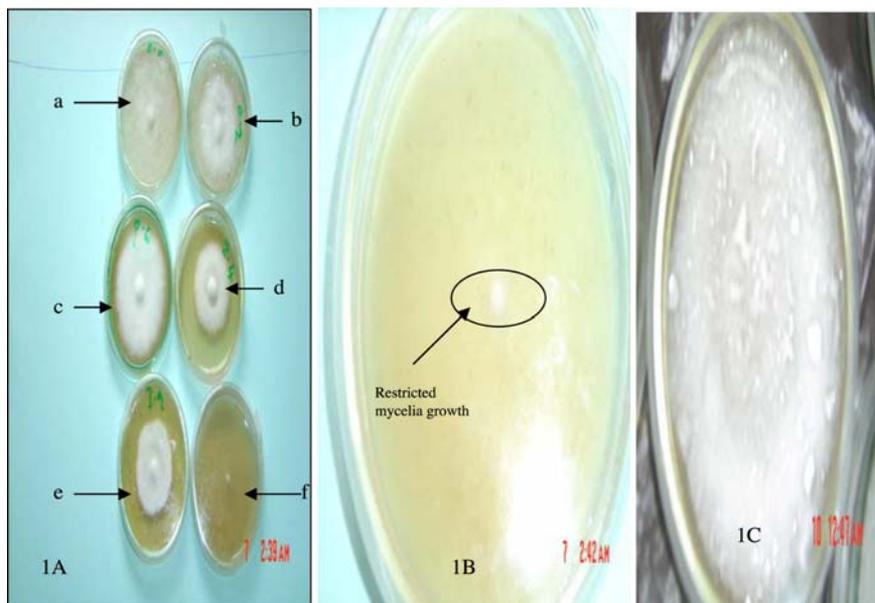
Effect of timing of application of crude garlic extract on the infection of *P. infestans*

Table 3 shows almost all treatments to have curative effects on development of *P. infestans*. Garlic extract at 2 % had significant (p

Table 1 Effects of different concentrations of crude garlic extract and synthetic fungicide (as positive control) and water (as negative control) on the percent inhibition of the fungus *P. infestans* in vitro in Holetta (Ethiopia) in 2004 crop season.

Concentration	Inhibition (%)
V- 8 Juice Agar (Water control)	0 g*
V- 8 Juice Agar with 0.5 % crude garlic extract	16f
V- 8 Juice Agar with 2 % crude garlic extract	38cde
V- 8 Juice Agar with 4 % crude garlic extract	41cd
V- 8 Juice Agar with 6 % crude garlic extract	40c
V- 8 Juice Agar with 8 % crude garlic extract	99.5ab
0.1 % Dithane M45 (Mancozeb 80 % WP)	100a

* Means followed with the same letter in the columns are not statistically different according to Duncan's multiple range test at $P < 0.05$ level.



Legend: a - 0% garlic crude extract concentration, b - 0.5% garlic crude extract Concentration, c- 2% garlic crude extract concentration, d - 4% garlic crude extract concentration, e - 6% garlic crude extract concentration and f -8% garlic crude extract concentration

Figure 1 **A** Effect of different percent concentrations of crude garlic extract on the growth of the fungus (*P. infestans*) at 18 days after inoculation; **B** The growth of the fungus (*P. infestans*) amended with 0.1% concentration of chemical fungicide (Dithane M 45) on the growth at 18th days and **C** The growth of *P. infestans* amended with water at 18th days after inoculation.

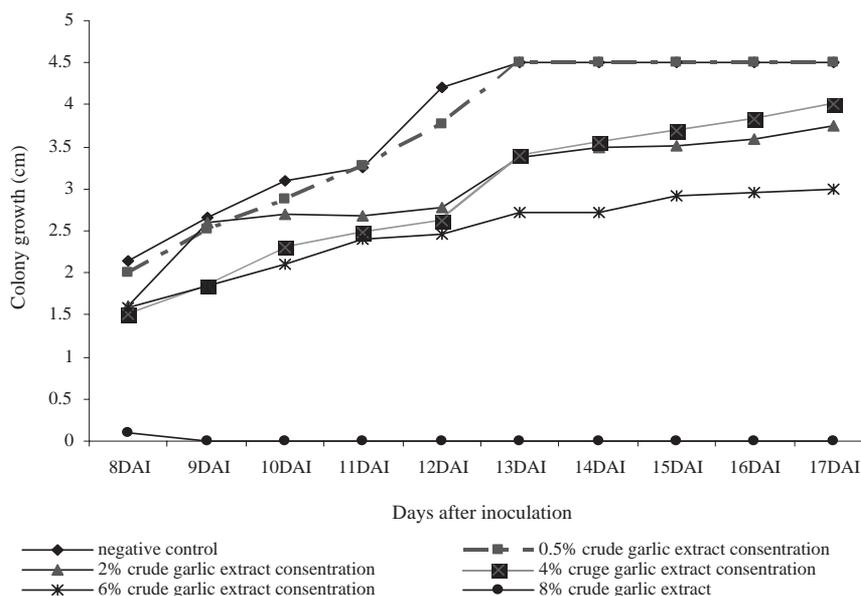


Figure 2 In - vitro mycelia growth of *P. infestans* in the course of 18 days after inoculation.

Table 2 Effect of different concentrations of crude garlic extract on the germination of Zoospore and sporangia of *P. infestans* in Holetta (Ethiopia) in 2004 crop season.

Concentration	Sporangia		Zoospore	
	Germ- nation	% Inhibition	Germ- nation	% Inhibition
V-8 Juice Agar with 0.5 % crude garlic extract	10	80	0	100
V-8 Juice Agar with 2 % crude garlic extract	0	100	0	100
V-8 Juice Agar with 4 % crude garlic extract	0	100	0	100
V-8 Juice Agar with 6 % crude garlic extract	0	100	0	100
V-8 Juice Agar with 8 % crude garlic extract	0	100	0	100
V-8 Juice Agar with 0.1 % Dithane M 45	0	100	0	100
V-8 Juice Agar (Water control)	75	0	72	0

< 0.05) influence and strongly inhibited the infection of *P. infestans* when it was applied 1 day earlier or at the same time of inoculation and showed 92 % and 100% inhibition effects in terms of lesion number and lesion size respectively. These degrees of inhibition obtained by applying 2 % crude garlic extract was comparable with the inhibition effect of the popular fungicide Dithane M 45 which gave 100% inhibition on lesion number and lesion size. But, when the times of application of the crude garlic extract were delayed by 2 to 3 days or applied 2 to 3 days earlier to inoculation, the inhibition effect was drastically and significantly ($p < 0.05$) reduced to 19% and 21, % or 29 % and 24 % respectively (Table 3). Of the main indicators for disease epidemic, the lesion number and lesion size in garlic extract sprayed treatments showed reliable performance over the negative control treatment. Mean lesion number inhibition in the garlic extract sprayed treatments had 75 % over the negative control treatment and less by 18 % from the positive control treatment. Mean lesion size was also reduced to 31 % compared to the negative control. The result showed that if the extract applied after infection took place, the efficiency of the extract highly reduced. This result suggested that somehow, the extract should be synchronized with the time the spore was landing on the host potato plant and

before the infection started.

DISCUSSION

In order to control potato late blight in a more sustainable manner and environmentally friendly, seeking for alternative materials like garlic instead of commonly used fungicides is getting much concern. Plant extracts and products provided major sources to realize this aim. Crude extract from garlic used in these experiments were significantly superior over the control to reduce the growth of the fungus, germination potential of zoospores and sporangia and to inhibit the infection of *P. infestans* by manipulating the time of application was possible. Though the studies were carried out in-vitro and green house, the results gave strong indications to the possibility to reduce disease infection in the potato production fields.

The result showed that mycelia growth of *P. infestans* was terminated even at lower concentration of crude garlic extract. This finding was in agreement with many workers who reported that garlic extract could inhibit the growth of pathogenic fungi (Applaton and Tanney, 1975; Bianchi *et al.*, 1997 and Wang *et al.*, 2004.) and of *P. infestans* (CAO and Arina, 2001).

In these observations, though it was

Table 3 Garlic crude extracts effect on the inhibition of *P. infestans* infection in vitro at Holetta (Ethiopia) in 2004 crop season.

Time intervals between treatment and inoculation	Dithane M 45, 0.01 % (Positive control)		Crude garlic extract 2 %		Water (Negative control)		
	L No ¹ .	Ls (cm) ²	% inhibition	L No	Ls (cm)	L No	Ls (cm)
3 days ahead	2	0.5	92ab*	21	2.1	26	3.3
2 days ahead	5	1.3	84abc	19	2.8	31	2.8
1 days ahead	0	0	100a	3	0.4	40	2.1
At the same time	0	0	100a	0	0	38	2.6
1 days after	7	1.2	81cde	17	1.8	29	3.0
2 days after	10	1.5	71d	29	2.6	35	2.4
3 days after	5	2.0	83abcd	24	3.2	29	3.1
Mean	4.14	0.14	87.37	16.14	1.84	65.14	2.7

¹Average number of lesions taken from the middle leaves of five plants. ² Average of lesions size taken from five plants and 5 lesions were measured from each plant.

under microscope, cells were totally distracted particularly when the concentrations were more than 2 % and in the case of lower concentrations some thickening of the cell walls were observed. Such phenomena in which the mechanism to stop any biological activity of the fruiting body of the fungus by using plant extracts were reported by Jacob and Serivastava (1982). Antifungal agents obtained from plants as extract act on the cell wall and lead to swelling and/or to destruction of the cells in the fruiting body. As a result the extracts terminate farther the processes of growth of the fruiting body of the fungus.

Garlic a natural food additive, one of the oldest components in folk medicine, it has been proved to be inhibitory to many fungi including *P. infestans* (Appleton and Tonney, 1975; Bianchi *et al.*, 1997). According to these results, the inhibition effect of the extract was effective when applied at a time of inoculation but when applied 2 to 3 days before or after inoculation the inhibition effect drastically declined. Though Othman *et al.* (1991) reported that, the crude antiphytoviral of garlic bubbilies was thermo-stable and the solution could keep its effectiveness after string for one week at 4°C, probably when it was exposed to external environment, possibly its effectiveness could deteriorate and it could lose its function after it was dried up for two days on potato leaves.

The results showed that if the extracts applied after infections, the efficiency of the extract drastically reduced. Although the number of lesions increased when the time spanned between inoculation and application of garlic increased, the lesion size measured was small compared to the size recorded in treatments where garlic extract was applied after inoculation. This probably meant that even the application of garlic was applied 2 to 3 days after inoculation and /or infection, it could contribute to the reduction of the size of the lesions which might have direct relation to the sporulation capacity of lesions. But generally application of the garlic extract prior to inoculation

was found to be more effective. This finding was agreed with the statement made by Carter and Locke (1994).

CONCLUSION

Significant ($P < 0.05$) inhibition effect on the growth of the fungus of *P. infestans* was obtained using all rates of concentrations. The result showed that mycelia growth of *P. infestans* was terminated even at lower (0.05 %) concentration of crude garlic extract and the highest concentration (8 %) was found comparable to the treatment amended with fungicide. Moreover, all the garlic extract concentrations tested showed their capacity to inhibit the germination of zoospores and sporangia.

These results also suggested that the extract was more efficient when it was applied before infection starts. Thus, time of application of the extract probably was a crucial factor in the use of the crude garlic extract to inhibit the infection. The findings of these study suggested crude garlic extract as a potential spray for the management of potato late blight disease. However in order to maximize its effectiveness and to make more efficient, it will be better to use the spray as one of the components in the integrated late blight management (IDM).

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