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In vivo evaluation of garlic (*Allium sativum*) extract in the control of potato late blight disease caused by *phytophthora infestans*

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Abstract

***In vivo* study was conducted to evaluate the efficacy of foliar application of garlic extract and two chemical fungicides (ridomil and z-force) in the reduction of late blight caused by *Phytophthora infestans*, increase of potato biomass and total tuber yield in natural field condition. The field experiment was conducted in Machambe village, Bokkos Local Government Area of Plateau State. Certified seeds were obtained from the National Root Crop Research Institute, Kuru, Plateau State, Nigeria. Potato seeds were grown in four row plots, 30-meter-long with spacing of 70cm between rows and 30cm within rows. Experiment was laid out in a Complete Randomized Block Design (CRBD) with four replications. Foliar sprays of 0.4 g/l Ridomil, Z-force and garlic extract respectively, at a 3day interval soon after first visible symptoms appeared to reduced disease incidence and resulted in higher biological and tuber yield compared to control (unsprayed plots). Ridomil and garlic extract were most effective in minimizing the disease incidence and producing better biological yields (33.95g and 31.02g) and tuber yields (15.93t/ha and 14.99t/ha) respectively. Control plot produced the least biological and tuber yield of 23.92g and 11.95t/ha respectively. There was no significant difference at P<0.05 between the tested treatments. There was significant difference in disease incidence reduction at (P<0.05) by the extract and chemical fungicides. Late blight incidence reduction effect of garlic extract was slightly higher than that of Z-force fungicide. Therefore, the evaluated plant material (garlic extract) could serve as an alternative to chemical fungicide in the control of late blight disease in potato.**

Keywords: late blight incidence, garlic, *Phytophthora infestans*, potato, *in vivo*.

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Introduction

Irish potato (*Solanum tuberosum*), major staple food crop after maize, rice and wheat (Fabeiro et al., 2001), belongs to the family Solanaceae and contributes to the UN's millennium development goals of providing food security and eradicating poverty (FAO, 2008). In developing countries, Irish potato is ranked first in energy production per hectare per day, significantly above cassava and cereals (FAO, 2008). It was introduced into Nigeria by European miners (WPA, 2006) and since has become an integral part of the farming system of the local farmers who are responsible for producing over 1,500,000 metric tons and 92% of Nigeria's annual output (NRCRI, 2010). Potato production is challenged by diverse pathogens; the most devastating is the *Phytophthora infestans* (Mont.) de Bary (causal agent of late blight). Late blight is a serious threat to potato production all over the world which not only causes severe crop damages but also contributes to considerable monitory losses due to excessive use of fungicides for its control (Fry & Goodwin, 1997). Late blight management has been heavily based on fungicide application and in many areas fungicide applications have increased over the last decade due to the introduction of new, more aggressive genotypes of the pathogen (Daayf et al., 2001). Even though synthetic pesticide use is a popular way of controlling pests because it is quick acting, and has direct results, public concerns have been raised over pesticide residues on fruits and food, effects on the environmental quality, the high cost of fungicide applications, and public pressures to minimize the use of chemical treatments requires alternative control measures such as natural plant products (Fry & Goodwin, 1997). According to Wang et al. (2004), natural plant products are known to have minimal environmental and health damage and affordable by poor farmers in contrast to chemical fungicides. 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 (Kassa et al., 2006). This study was conducted to compare the effect of garlic extract, ridomil and z-force on late blight incidence reduction, increase biomass and total tuber yield of the potato crop as well as promote alternative control measures of the fungus and provide information on the use of garlic extract to control the pathogen in Jos, Plateau State, Nigeria.

Materials and methods

Experimental site and design: The field experiment was conducted in Machambe village, Bokkos Local Government Area of Plateau State, Nigeria. Certified seeds were obtained from the National Root Crop Research Institute, Kuru, Plateau State, Nigeria. Potato seeds were grown in four row plots, 3-meter-long with spacing of 70cm between rows and 30cm within rows. Experiment was laid out in a Complete Randomized Block Design (CRBD) with four replications.

Preparation of plant extract: Bulbs of fresh garlic were purchased from the local markets. The bulbs were washed with sterile water, peeled and cut into pieces. 250g was kept for an hour at room temperature (25-28°C), ground using mortar and pistil. It was then soaked in 11% ethanol for 48hours after which it was filtered through Whatman

No. 1 filter paper, concentrated into paste and stored at 4°C in the refrigerator until ready for use as describe by Majeed et al. (2011). 0.4g was weighed out and diluted in 1liter of sterile water to obtain 0.4g/l (Sallam & Kamal, 2012). Two commercial chemical fungicides (Ridomil and z-force) were used. 750g of each fungicide was purchased from the market in sachets. 0.4g was measured out and dissolved in 1litre of distilled water to obtain 0.4g/l.

Application of plant extract and fungicides *in vivo*: The prepared plant extract and synthetic fungicides were applied using a 20litres capacity knapsack sprayer to potato leaves at 0.4g/l concentration. The treatments were applied at a 3-day interval soon after the late blight symptoms were visible at early hours of the day (morning time when humidity is high), being the conventional method of fungicides application in the area of study. No spraying was done on the control plot. (Majeed et al., 2011).

Disease incidence and severity (%): Disease incidence and severity were assessed on the leaves of the plant weekly. Disease incidence of late blight was assessed by counting the number of diseased leaves per plant. This was done on a scale from 0-5 (Mantecon, 2009). The observation started from first appearance of symptoms on leaves continued until the downfall of observed leaves. The scale was as followed; 0 = healthy (0%), 1= First foliar symptoms present and no defoliation, 2= up to 25% defoliation plus foliar blight, 3= up to 50% defoliation plus foliar blight, 4= up to 75% defoliation plus foliar blight and 5= 100% defoliation. Disease severity was calculated and the objective was to have readings at low, medium and high levels of disease in all plots. The Area Under Disease Progress Curve (AUDPC) was then estimated using the formula derived from Forbes et al. (2014) as follows:

$$\text{AUDPC}=\sum_{i}^{n-1}\frac{(Y_{i}-Y_{i+1})}{2}\left(t_{i+1-t_{i}}\right)$$

Where, Y_i = disease severity at i^{th} observation, t_i = time (days) from i^{th} observation, n = total number of observations.

Results and Discussion

Late blight disease incidence was assessed weekly for a period of 12weeks after planting (Table 1). Disease incidence increased with increase in days (Plate 1). The least disease incidence of 29.17% was seen for Ridomil followed by garlic which had mean disease incidence of 32.92%. The highest mean disease incidence of 47.92% was seen for control. Statistical analysis showed that the disease incidence for control was significantly higher than all the test treatments (Ridomil, Z-force and garlic) and also showed that the disease incidence for Ridomil, Z-force and garlic was not significantly different from each other at P>0.05 level of probability.

Table 1: Effect of Garlic extract, Ridomil and Z-force on the incidence of late blight of potato.

Weeks	Control	Garlic	Ridomil	Z-force	Means
WK 1	0	0	0	0	0.00^{f}
WK 2	0	0	0	0	0.00^{f}
WK 3	0	0	0	0	0.00^{f}
WK 4	10	0	0	0	2.00^{f}
WK 5	40	10	10	20	16.00^{e}
WK 6	40	15	20	20	19.00^{de}
WK 7	60	20	20	20	24.00^{d}
WK 8	60	40	20	50	34.00^{d}
WK 9	80	60	60	60	52.00^{bc}
WK 10	85	75	60	70	58.00^{b}
WK 11	100	80	65	90	67.00^{b}
WK 12	100	95	95	100	78.00^{a}
MEANS	47.92^{a}	32.92^{b}	29.17^{b}	35.83^{b}	

Means with same alphabets are not significantly different at P>0.05.

The highest biomass and tuber yield of 33.95g and 15.93t/ha respectively were recorded for Ridomil with average disease incidence of 53.33%, followed by garlic with biomass and tuber yield of 31.02g and 14.99t/ha respectively and the lowest disease incidence (53%), and z-force with biomass and tuber yield of 30.02g and 13.49t/ha and average disease incidence of 60%. The lowest biomass and tuber yield were recorded for control with 23.92g and 11.95t/h respectively and had the highest average disease incidence of 73.33%. Plate 2 shows biomass and total tuber yield for the different treatments as seen in figure 1. Figure 2 shows the disease severity curve.

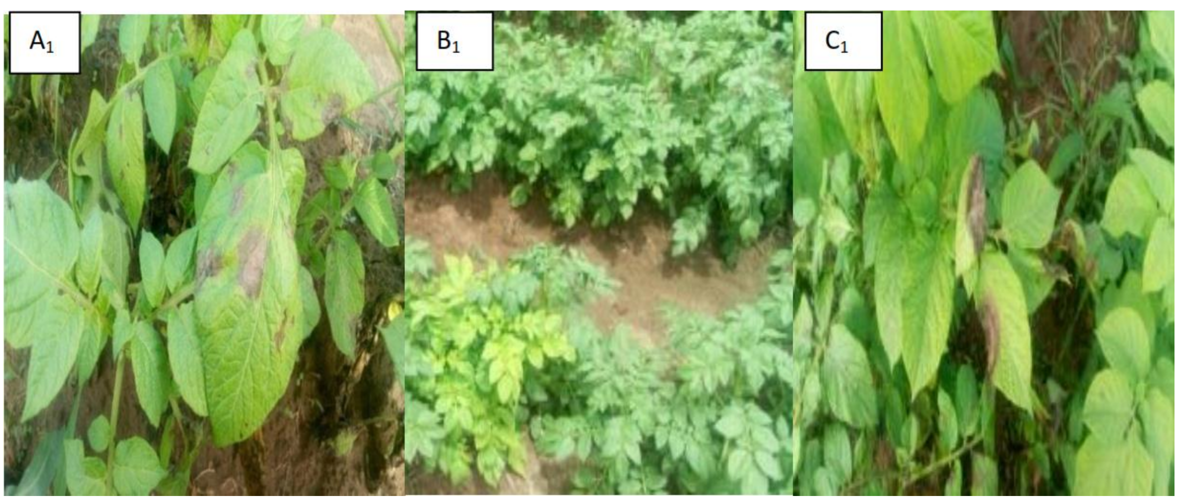

Plate 1: Photos of disease incidence. A_1= First disease incidence at 5weeks after planting on Garlic treated plants, B_1= First disease incidence at 5weeks after planting on Ridomil treated plants, C_1= First disease incidence at 5weeks after planting on Z-force treated plants, A_2= Elaborated disease incidence at 12weeks after planting on Garlic treated plants, B_2= Elaborated disease incidence at 12weeks after planting on Ridomil treated plants, C_2= Elaborated disease incidence at 12weeks after planting on Z-force treated plants.

The disease severity assessment revealed that control plot which had no treatment showed the highest level of disease severity with AUDPC value of 3641. The lowest severity level (2118) occurred in plot treated with Ridomil followed by garlic (2434) while Z-force had 2660 severity level, suggesting that all the test treatments lowered disease incidence better than control.

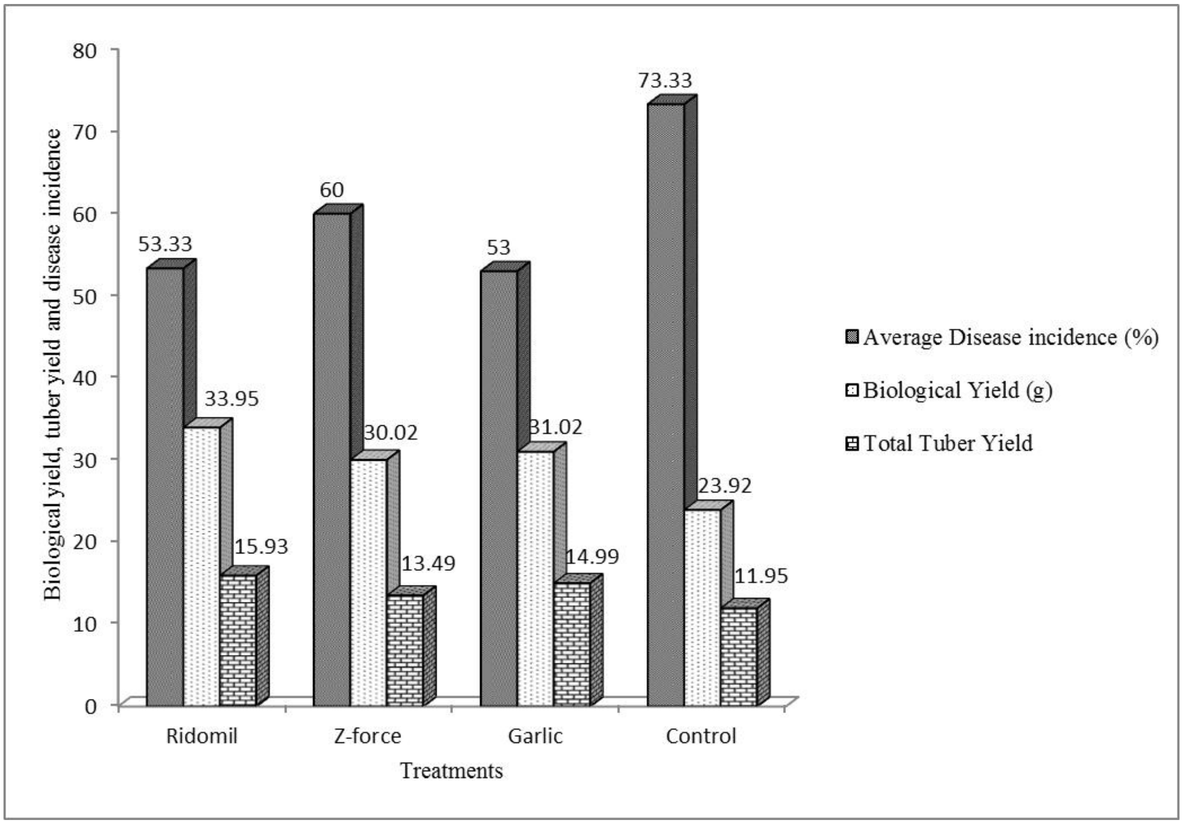

Figure 1: Biological yield and tuber yield as affected by potato late blight disease incidence.

In order to control potato late blight in a more sustainable and environmentally friendly manner, the search for alternative materials like garlic instead of the commonly used chemical fungicides is gaining much concern. The extract of garlic is of great use in different areas such as medicine, agriculture and cosmetics to mention but a few. The effect of garlic extract, ridomil and z-force fungicides on reduction of late blight disease incidence, increase in both biomass and total tuber yield were evaluated *in vivo* and the results gave strong indications to the use of the garlic to reduce disease infection in the potato production fields. The *in vivo* study showed a considerable and comparable inhibition of the garlic extract to the two chemical fungicides tested against late blight disease of potato. Both the garlic extract and chemical fungicides lowered disease incidence significantly above control. At 11 weeks after planting, there was a 100% late blight incidence on control plots (un-sprayed plots) whereas, plots treated with garlic extract, ridomil and z-force had late blight incidence of 80, 65, and 90% respectively. Figure 1 showed that biomass and tuber yield decreased with increase in disease incidence.

Plate 2: Biomass and total tuber yield for the different treatments. A and E= Tubers from control plot in storage and field respectively, B and F= Tubers from Z-force-treated plant in storage and field respectively, C and G=Tubers from Garlic extract-treated plant in storage and field respectively, D and H=Tubers from Ridomil-treated plant.

However, the application of garlic extract, ridomil and z-force fungicides significantly reduced late blight incidence by 32.92%, 35.83% and 29.17% respectively, which corresponded to better biological and tuber yields compared to control. Better biological yield and larger tuber sizes on plots treated with garlic and chemical fungicides could have resulted from the reduced disease incidence which may have increased the photosynthetic leaf area and greater amount of photosynthate being transferred to tubers for storage (Olanya et al., 2001). Lowered disease incidence by garlic extract may be attributed to inhibitory effects of bioactive compounds such as allicin (Rahman & Motoyama, 2000). This is in agreement with previous reports by Kassa et al. (2006) which described allicin as bioactive compounds from *Allium sativum*. This could have increased photosynthetic activity of leaves resulting in better yield of potato crops. The *in vivo* results from this study generally showed that there was high late blight incidence at the site of experiment (Bokkos). This however, could have been due to the weather condition occurring at the time of the research which favoured the growth of *P. infestans*. The experimental site is located on the edge of the Jos Plateau characterized by deep gorges and misty weather. This misty weather may have been responsible for the relatively high incidence and equally high severity of the reported late blight disease. Another probable factor that may have been responsible for the severe incidence of late blight in this study was the heavy rainfall which came very early in the season (late March and April).

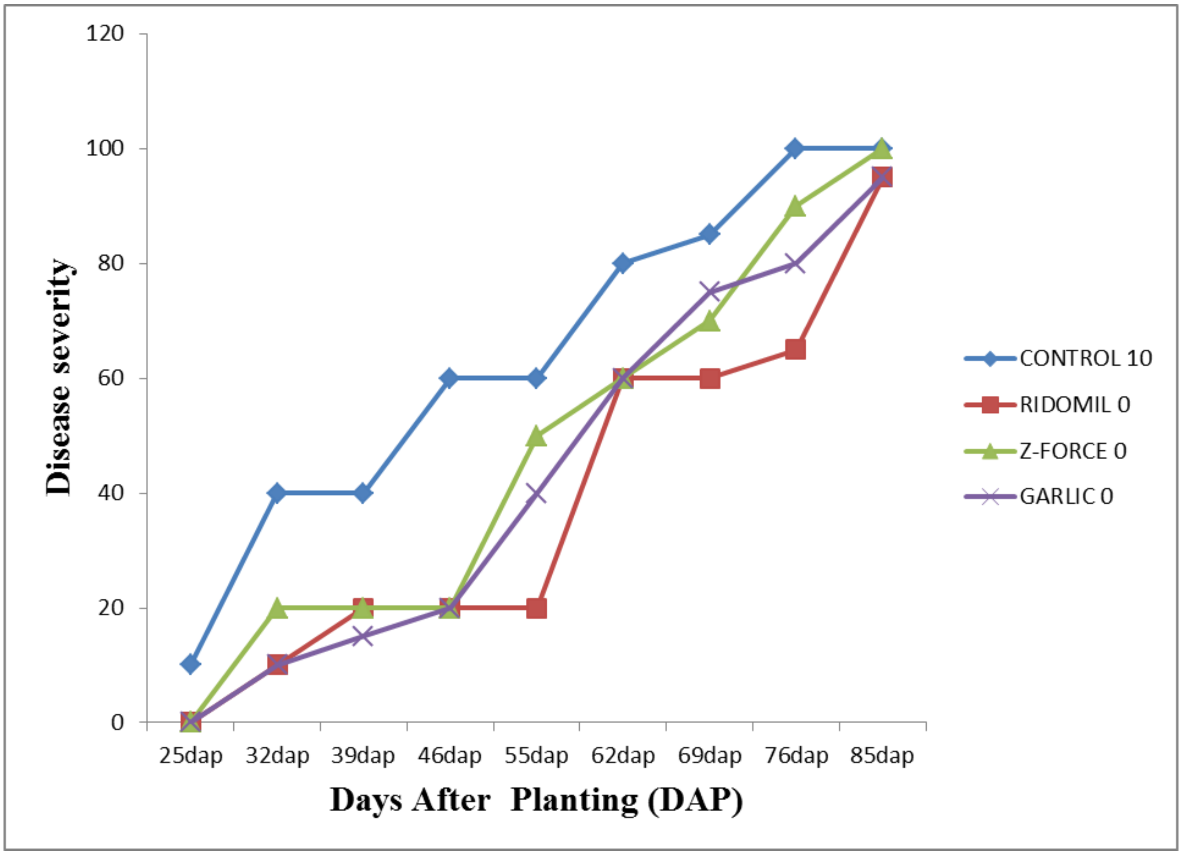

Figure 2: Disease severity curve of potato late blight disease after treatment.

Heavy rainfall had been reported to be a major factor in accelerating the incidence and severity of potato late blight most especially in the tropical highlands (NRCRI, 2012; Hijmans et al., 2000). In this study, cultivation of the potato crop started with the earliest rainfall which also coincided with a period of unusually high temperature. This situation may have resulted in the potato blight outbreak early in the growing season before the commencement of tuberization by the potato crop plants. The origin of the primary inoculum responsible for the high disease incidence is not certain, but it could probably be traced to the regular and usual practice of mono-cropping around the experimental site. This is in line with the report by Bouws and Finckh, (2008) who stated that growing of potato along with a non-host crop plant species reduces the spread of potato late blight pathogens significantly. The *in vivo* study revealed that there was no significant difference in the inhibition of *P. infestans* amongst the conventional fungicides and the evaluated garlic extract, and all tested treatments showed significant disease reduction over control. Statistical analysis revealed that the biomass and total tuber yield for all test treatments were significantly greater than control but not significantly different from each other at P>0.05 level of significance. These results suggested garlic extract as a potential spray for the management of late blight disease of potato. However, in order to maximize its effectiveness and make it more efficient, it may be better to use the spray (garlic extract) as one of the components in the integrated late blight management.

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