academicJournals

Vol. 8(37), pp. 4541-4545, 19 September, 2013
DOI:10.5897/AJAR11.259
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effects of aqueous extract of *Ricinus communis* on radial growth of *Alternaria solani*

Bayaso I.[1]*, Nahunnaro H.[2], and D. M. Gwary[3]

[1]Ministry of Agriculture headquarters P. M. B. 2079, Yola, Adamawa State, Nigeria.
[2]Department of Crop Production and Horticulture, Federal University of Technology P. M. B. 2076, Yola, Adamawa State, Nigeria.
[3]Department of Crop Protection, Faculty of Agriculture, University of Maiduguri, P. M. B. 1069, Maiduguri, Borno State, Nigeria.

Accepted 8 August, 2013

The study evaluated the effect of *Ricinus communis* aqueous extract on radial growth of *Alternaria solani* and its most effective concentration. The experiment was laid out using a Completely Randomized Design (CRD) with *R. communis* extract tested at 3 concentrations of 25, 50 and 100% plus control on Potato Dextrose Agar (PDA) amended medium in 3 replications for 2 separate experiments. Isolation and identification of the early blight pathogen was made by symptoms on tomato plants, macro and microscopic observations in pure culture. Data on radial growth were collected, statistically analysed and percent inhibition (I%) was calculated. Results revealed that, *R. communis* at 100% concentration recorded the lowest radial growth either at 24, 48 and 72 h post inoculation (hpi) in the first, second, and combined results. The combined results further revealed that, the lowest radial growth 1.43, 2.00, and 2.72 cm were recorded in *R. communis* treatment at 24, 48 and 72 hpi, respectively. I% varied from 26 to 59%, according to the experimental conditions, and it was then concluded that, *R. communis* extract used at different concentrations had inhibitory effect on *A. solani*. It is was then suggested that, *R. communis* at 100% concentration could be put to field trial to evaluate its effectiveness in the control of the early blight pathogen *A. solani*.

Key words: *Alternaria solani, Ricinus communis*, radial growth, concentration.

INTRODUCTION

Tomato is attacked by many diseases which constitute a serious setback to its production (Peet, 2003). Some of these diseases include: early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), damping off (*Phythium solanacerum*), bacterial wilt (*Burkholderia solanacearum*), fungal wilt (*Fusarium oxysporum*) and nematode (*Meloidogyne javanica*). The early blight of tomato induced by *A. solani* (Ell. and Mart.) has in no small measure contributed to yield losses of up to 79%, as reported from Canada, India, USA, and Nigeria (Sherf and MacNab, 1986; Gwary and Nahunnaro, 1998).

In the past, growers reported increasing incidence of this disease and decline in its control and have to deal with development of resistance of *Alternaria* species toward over-use of fungicides (Iacomi-Vasilescu et al., 2004). Hence, systematic screening of plant extracts may result in the discovery of novel effective compounds (Tomoko et al., 2002). Therefore scientific research is important in order to determine the presence of antifungal activity in the crude extracts of some common plants, known or not for their biological activity. Consequently, this study was aimed at evaluating *Ricinus communis*

*Corresponding author. E-mail: ibayaso@gmail.com.

extract in control of the early blight pathogen *A. solani in vitro* and at determining the most effective concentration.

MATERIALS AND METHODS

Experimental sites

This study was carried out in the Laboratory of the Crop Production and Horticulture Department, Federal University of Technology, Yola, Adamawa State, Nigeria which lies between latitude 8°N and 11°N and longitude 11.5°E and 13.5°E.

Experimental design and layout

The experiment was conducted in the laboratory using a Completely Randomized Design (CRD) and aqueous plant extracts of Castorbean plant *(R. communis)* used at 3 concentrations (25, 50 and 100%) with a control to give a total of 4 treatments.

Collection of plant materials and preparation of extracts

R. communis plant was collected from around the Federal University of Technology, Yola. The collected plant materials were rinsed, washed with 10% sodium hypochlorite (NaOCl), air dried and later packed in brown envelops and oven-dried at 70°C for 20 mins according to Akinbode and Ikotun (2008). Thereafter, the plant materials were grinded using mortar and pestle, sieved using 40 mm sieve. About 200 g of the plant powder was added into 500 ml of distilled water and stirred to get uniform suspension. The suspension was allowed to stand for 24 h and the content was filtered using a muslin cloth and kept in glass bottles until needed. The different concentrations were then prepared by taking 25, 50, and 100 ml of the stock preparation and dissolved in distilled water to give 25, 50, and 100% concentrations.

Isolation and identification of *A. solani*

Diseased tomato leaves with the symptoms of *A. solani* were collected from farms around the University. A small piece from the advancing margin of a lesion on diseased leaf was cut with a sterile pair of scissors sterilized with 10% NaOCl (Larone, 1995). The tissues were then washed thoroughly in several changes of sterile distilled water and placed aseptically into 9 cm diameter Petri dishes containing 15 ml of sterile PDA. The plates were incubated for 7 days at room temperature (28 to 30°C). Distinct colonies on the plates were selected, purified by repeated culturing and maintained on PDA slants. The fungus isolated was identified as *A. solani* using the macroscopic and microscopic identification guide according to Larone (1995).

Preparation of growth medium and inoculation

About 39 g of PDA powder (Sigma GMBH) was dissolved in 1000 ml of distilled water and the content was stirred and autoclaved for 25 mins at 115°C (Awale, 2001). The medium was allowed to cool down and was then aseptically poured into 25 ml flavour bottles. Thereafter, 5 ml of the plants extract prepared was poured into the Petri dishes. About 15 ml of molten PDA at 45 to 50°C was poured aseptically onto the plant extract in the Petri dish and swirled round 5 times for even dispersion of the extract into the agar and allowed to solidify, before the pathogen was inoculated (introduced) into the middle of the 'poisoned agar'. A mycelial plug of 5 mm diameter from 3 days old fungus was cut using a 5 mm sterile cork borer and transferred to the PDA plate in the center of the Petri dish and was kept in a sterilized fume cupboard kept at room temperature of 28 to 30°C.

Data collected

Radial growth

Measurement of the radial growth in centimeters (cm) was done and the radial growth was determined by using the formula K_r according to Reeslev and Kjoller (1995):

$$\text{Radial growth } (K_r) = \frac{(R_1 - R_0)}{(t_1 - t_0)}$$

Where, R_0 and R_1 are the colony radii at time t_0 and t_1 respectively, determined after 24, 48 and 72 h from inoculums.

Inhibition percentage

The inhibition percentage was calculated measuring the radial growth of the fungus grown on control and amended plates after 24, 48, and 72 hpi, using the following formula (Harlapur et al., 2007):

$$I\% = \frac{100 \times (C - T)}{C}$$

Where, I% = inhibition percentage of pathogen growth, C = average radial growth in control plates and T = average radial growth in plates amended with *R. communis* extract suspension.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) for a completely randomized design using SAS (1999) statistical package. The means were separated using the Least Significant Test (LSD) at $P = 0.05$.

RESULTS

Radial growth of *A. solani* at 24, 46, and 72 h

Results of the first laboratory experiment at 24, 48 and 72 h shows that, the lowest radial growth was recorded in *R. communis* 100% concentration with means of 1.57, 2.16, and 2.88 cm (Plate 1) with the control recording the highest radial growth of 3.53, 4.09 and 4.59 cm (Plate 2). Also in the second experiment the treatment means showed that, *R. communis* at 100% concentration recorded the lowest radial growth with 1.30, 1.83, and 2.55 cm at 24, 48 and 72 h. In addition the control recorded the highest radial growth with mean of 3.40, 3.98 and 4.43 cm at 24, 48 and 72 h (Table 1).

The combined results in Table 2 revealed that, at 24, 48, and 72 h *R. communis* at 100% concentration had the lowest means of 1.43, 2.00, and 2.72 cm, followed by 50% concentration with 1.83, 2.32, and 2.93 cm. The control recorded the highest radial growth of 3.47, 4.04 and 4.51 cm at 24, 48, and 72 h.

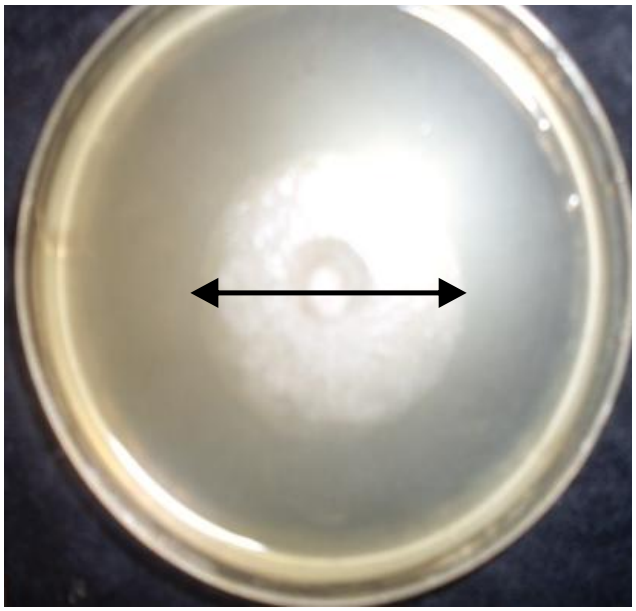

Plate 1. The lowest radial growth in *R. communis* 100% (72 h).

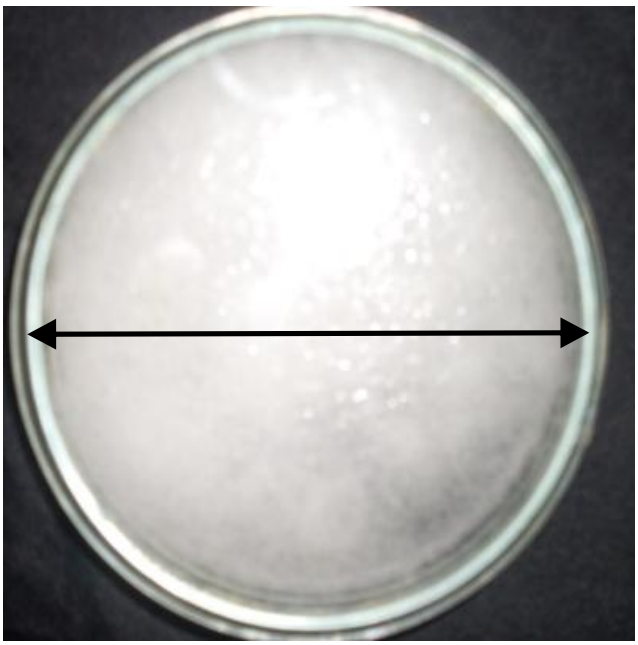

Plate 2. The highest radial growth in control (48 h).

Inhibition percentage of radial growth of *A. solani* at 24, 48 and 72 h

The I% for the combined results (Table 3) revealed that, *R. communis* extracts inhibited radial growth in the range of 41 to 59% at 24 h in comparison to the control. At 48 h, the result indicated that, *R. communis* extracts inhibited fungal radial growth in the range of 33 to 51%. At 72 h, the result of the calculated I% showed that, *R. communis* extracts showed inhibitory effect on the radial growth in the range of 26 to 40% as shown in Table 3.

DISCUSSION

In this study, screening of the *in vitro* effect of *R. communis* aqueous extract for antifungal activity against *A. solani*, responsible for early blight of tomato, was carried out at 3 concentrations supplied to PDA medium. Results of the effects of plant extract on radial growth of *A. solani in vitro* revealed that, *R. communis* extract was able to reduce the radial growth and at the end of the experiment (72 h) the percentage at 100% concentration was still 40%. This may be attributed to the presence of toxic compounds, like ricin and ricinine in the extracts, as reported by Ukpabe (2002) who stated that, leaf extracts of *R. communis* inhibited the growth of *F. oxysporum*. Comparing the rate of radial growth in medium amended with *R. communis* aqueous extract with that of the control; it could be deduced that, the pathogen grew freely on the control medium, establishing itself and using up the food, while on the “poisoned food” of PDA containing the plant extract, growth was significantly reduced. The inhibitory effects of the extracts might be due to the chemicals present in the plant. Ricin is a proteinaceous toxin as reported by Lowery et al. (2007). This therefore suggests that, the plant extracts of *R. communis,* posses inhibitory effects on the growth of the fungus. Similarly the seeds, leaves, and stems of the plant contain ricin and ricinine, dihydroxystearic, linoleic, oleic, and stearic acids, β-sitosterol (Oplinger et al., 1997).

Tariq (2009) also reported that, growth in fungi is affected by the availability of substrate. He further stated that, the growth of most fungi is rapid at the exponential phase until one or more nutrients become limiting or depleted and/or metabolic products accumulate to low level. This could explain why the percentage was higher at 48 h than at 72 h in comparison with the control, since the fungus already had occupied all the surface of PDA plates and ceased to grow.

Similar results on extracts from five Chinese medicinal herbs were reported by Tongle et al. (2002) in their study: *Galla chinensis, Rheum palmytum* (root), *Sophora flavescens* (root), *Terminalia chebula* (fruit), and *Magnolia officinalis* (bark) showed inhibitory effects against *Phytophthora infestans in vitro* by inhibiting sporangia germination, mycelial growth and/or infection on potato leaves.

In this study, results on the plant extracts showed that, *R. communis* at 100% concentration inhibited radial growth by 40 to 59% in the combined results. This finding concurs with that of Baldrian and Gabriel (2002) who reported that, *Piptoporus betulinus* growth was found to be concentration-dependent as fungal growth was inhibited at higher concentration. This could be inferred that *R. communis* could inhibit the growth of *A. solani*. In

Table 1. Effects of plant extracts on radial growth (cm) of *A. solani* first and second experiments.

Treatment	Hours					
	24		48		72	
	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment
†Ricom25	2.27	1.85	2.66	2.76	3.35	3.33
Ricom50	1.75	1.91	2.43	2.20	2.95	2.90
Ricom100	1.57	1.30	2.16	1.83	2.88	2.55
Control	3.53	3.40	4.09	3.98	4.59	4.43
Mean	2.28	2.12	2.84	2.70	3.44	3.30
LSD	0.53	0.11	1.04	0.24	0.84	0.18
Prob. F	**	**	**	**	**	**

†Ricom = *Ricinus communis*; **, highly significant (*P* = 0.01)

Table 2. Combined means of the effect of *Ricinus communis* extract on radial growth (cm) of *Alternaria solani*.

Treatment	Hours		
	24	48	72
Ricom† 25	2.06	2.71	3.34
Ricom50	1.83	2.32	2.93
Ricom100	1.43	2.00	2.72
Control	3.47	4.04	4.51
Mean	2.20	2.77	3.38
LSD	0.27	0.47	0.50
Prob. F	**	**	**

**, Highly significant (*P* = 0.01).

Table 3. Combined results of the effect of *A. solani* on inhibition percentage (%) on radial growth of *A. solani.*

Treatment	Concentration (%)	% I (hpi)		
		24	48	72
Control	0	0	0	0
Ricom	25	41	33	26
Ricom	50	47	43	35
Ricom	100	59	51	40

addition, the fungus is much more sensitive to *R. communis* at higher concentration in affecting radial growth at lower concentrations.

Conclusion

The plant extracts at different concentrations tested on *A. solani* showed promising prospects for its utilization in plant disease control. The results showed that, *R. communis* at 100% concentration could reduce radial growth and inhibit growth Therefore, plant extracts are a potential source of botanical fungicides, after successful completion of wide range of field trials.

RECOMMENDATIONS

Based on the findings of this study, it was recommended that, *R. communis* at 100% concentration should be put to field trail to confirm its effectiveness.

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