academicJournals

Vol. 13(17), pp. 1791-1795, 23 April, 2014
DOI: 10.5897/AJB2014.13744
Article Number: 7CAA12544272
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Management of foliar and soilborne pathogens of cowpea (*Vigna unguiculata* L. Walp) with two garlic varieties (*Allium Sativum* A. Linn)

Peluola Cecilia[1]* and Fadina Olubunmi[2]

[1]Department of Botany and Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.
[2]Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received 20 February, 2014; Accepted 12 March, 2014

White and pink garlic extracts were tested for their antifungal potentials on mycelial radial growth, spores and sclerotial production of *Macrophomina phaseolina* (Tassi) Goid*,* *Colletotrichum destructivum* O gara and *Colletotrichum capsici* (Syd) Butler and Bisby pathogens of cowpea *in vitro*. Water or ethanol extracts of common pink and common white garlic varieties were tested at a concentration of 250 ppm while sterile distilled water served as control. *In vivo* study was based on white garlic extract alone at 0, 50 and 100% concentrations on *M. phaseolina* and *C. capsici.* Data were subjected to ANOVA and means was separated at P=0.05. Water extracted white garlic gave over 90% inhibitions of mycelial growth of *M. phaseolina* and gave higher inhibitions than water or ethanolic pink garlic extracts on all the three pathogens. It is not significantly lower P=0.05 than conventional fungicide benomyl at 0.5 gai/kg. However, *in vivo* result was phytotoxic to cowpea seeds at 100% concentration of white garlic extract. On cowpea variety TVx 3236, *C. capsici* inoculated seed germination and pathogen control was 100%, also, *M. phaseolina* inoculated seeds germination was 100% but there was no pathogen control at 50% garlic extract. In contrast, cowpea variety IT84S-2246-4 seed germination was 77% when treated with *M. phaseolina* and 100% with *C. capsici* but reduced pathogen control at 50% garlic extract.

Key words: Pink and white garlic, fungal pathogens, conventional fungicide, cowpea varieties, germination, phytotoxicity, control.

INTRODUCTION

The use of synthetic agro-chemicals in developing countries to reduce pests and diseases present serious environmental problems to growers especially peasant farmers who depend on agriculture for sustenance and livelihood. Most often, the response of increasing demand for food has always been in form of increased use of synthetic agro-chemicals. These chemical pesticides are often poorly handled, resulting in the contamination of the environment. Globally, there is increasing demand in favor of natural pesticides among consumers. Peasant farmers may

*Corresponding author. E-mail: peluola@yahoo.ca or gola2k4@yahoo.com.

also find this approach more acceptable due to the fact that it ease the part of accessibility in their local environment and it flow freely with their normal way of life. Nature herself has offered us a profusion of plants for use in natural crop protection for a cleaner and safer environment. One of such plants is *Allium sativum* (garlic). This plant is widely grown in Nigeria and it is believed to be antimicrobial and antifilarial. Traditionally, *A. sativum* has been used in the treatment of worms and dysentery in children and adults. In the kitchen, garlic is used for seasoning. There are several varieties of garlic but the most popular are the common white and the common pink (Ngeze, 2001).

Cowpea (*Vigna unguiculata* (L). Walp) is a legume of preference in the tropic where it supplies millions of peoples as their means of livelihood as food, fodder and cash. Disease is one of the important constraints to maximum production. All stages of growth as well as different parts of the plant is attacked by pests and diseases. Important foliar fungal pathogens of cowpea in Nigeria are anthracnose and brown blotch caused by *Colletotrichum destructivum* and *Colletotrichum capsici*. (Emechebe and Shoyinka, 1985). Ashy stem and charcoal rot diseases occurred sporadically on cowpea and are caused by seed and soil-borne *Macrophomina phaseolina* (Tassi) Goid, a pathogen of variable host (Emechebe and Shoyinka, 1985). Its ability to colonize several hosts and sporadic occurrence makes it more threatened. Over 500 hosts had been reported for *M. phaseolina* while loss from soybean field alone was estimated to be 77% in the USA (Hewitt, 1988). Although, benzimidazole based compounds are available against these pathogens; risk of fungicide resistance, the scale of economy and wrong attitude of consumer to chemical handling makes them less practicable for peasant farmers. Alternative methods that are cheaper, environmental friendly, biodegradable, easy to prepare and apply are desirable. Hence, the major objective of this study is to explore the use of garlic extracts in the control of these pathogens.

MATERIALS AND METHODS

Fresh garlic was purchased from an open market in Ibadan, Oyo state, Nigeria. The garlic lobes were washed and air-dried on the laboratory bench. 25 g each of the common white and common pink garlic were weighed separately and blended for 4 min with commercial warring blender and made up to 250 ml with sterile distilled water. The suspension was transferred into a beaker and wrapped up with aluminium foil. It was steam-boiled at 60°C for 2 h to enhance the extraction of the active components. The resulting solution was decanted and filtered with Whatman® filter paper No. 1. Sterilization of the water extract was obtained by passing the solution through 0.45 µm millipore filters. Ethanol extraction was carried out using the same method but was concentrated to about 2 ml with rotary evaporator and left to air-dry so as to remove any traces of ethanol. 250 mL of sterile distilled water was added to re-dissolve the concentrated extract, which served as the stock. Sterile distilled water was used as control.

Isolation of the test pathogens

The *M. phaseolina* fungal isolates used were isolated from germinating cowpea variety IT84S-2244-6 grown on infected soil. The two species of *Colletotrichum* (*C. destructivum* O gara*, C. capsici* (Syd) Butler and Bisby used for these studies were isolated from IITA cowpea demonstration field, IITA Ibadan, Nigeria. Blotter method as described by Waller et al. (1998) was employed during the study.

Fungitoxicity test *in vitro*

Antifungal potential of the extracts were tested on these pathogens using acidified potato dextrose agar (APDA). One milliliter (1 ml) of each extract was dispensed into 9 cm - diameter Petri dish. 3 cm mycelia disc taken from the margin of 7 days old cultures of test pathogens viz.- *C. destructivum C. capsici* and *M. phaseolina* was placed at the centre of each Petri dish containing the extracts and molten APDA. The plates were incubated at 28 to 30°C for 7 days. Fungitoxic effects (fungicidal and fungistatic) were observed daily, beginning at day 2 until day 7 after inoculation and incubation. Benomyl, a conventional fungicide at 0.5 g ai/litre of water and sterile distilled water served as positive and negative control, which was set up without the plant extracts. Colony diameter was measured after 7 days as the mean growth along two axes on two pre-drawn perpendicular lines on the reverse side of the plate. Percentage fungitoxicity were expressed according to Awuah (1989).

$$MP = [(M1 - M2) / M1] \times 100$$

Where: MP = percentage inhibition of mycelial growth, M1 = Mycelial growth in control Petri dish without extract or fungicide, M2 = Mycelial growth in extract/fungicide Petri dish.

Control of artificially inoculated cowpea seed with garlic extract

Approximately 300 g seeds of cowpea variety TVx 3236 and IT84S-2246-4 each was surfaced sterilized with 0.5% NaCl for 1 min, air-dried in the laminar flow and treated with garlic extract by soaking the seed in the extract for 3 mins at 50 and 100% concentrations. Cowpea seeds were later inoculated separately with the pathogens at 4.0×10^6 conidia/ml of *C. capsici*. The inoculum meal of *M. phaseolina* was used to coat cowpea seeds completely by scraping aseptically the surface of a fully grown (5 day old) mycelial mat of *M. phaseolina*, marched together and two drops of Tween 80® was added to enhance adhesiveness to the treated seeds. The cowpea seed were planted on three seeds per pot to a depth of 3 cm in a heat-sterilized soil. Observations on damping-off symptom (pre- and post- emergence), seedling growth and disease severity were observed for 21 days after inoculation and planting. A control treatment was set up with sterile distilled water.

Statistical analyses

All experiments were repeated twice. Data were analyzed using generalized linear model (GLM) procedure of statistical analysis software (SAS, Institute 2001). Means comparison were carried out at P = 0.05.

Table 1. Inhibition of mycelia radial growth (%) of three fungal isolates treated with pink or white garlic extracts and compared with benomyl.

Pathogen	Pink garlic 250 ppm at 7DAI		White garlic 250 ppm at 7DAI		Conventional fungicide
	Water	Ethanol	Water	Ethanol	Benomyl 0.5 gai/Kg
M. phaseolina	72.0[a]	65.1[a]	91.5[a]	82.6[a]	90.0[a]
C. destructivum	36.0[b]	34.2[b]	41.5[b]	33.39[b]	100.0[a]
C. capsici	22.0[c]	27.7[c]	54.1[a]	34.45[b]	100.0[a]

DAI= Days after inoculation; Means followed by the same letter within a column are not significantly different P=0.05.

RESULTS

At day 4 after inoculation, the *in vitro* result gave 100% inhibition of the mycelial radial growth, conidial and sclerotial germination of the three pathogens treated with 250 ppm of both pink and white garlic extracts. However, at 5 days after inoculation, pink or white garlic extracted in ethanol generally gave reduced inhibition relative to garlic extracted with water till seven days after inoculation. Petri dishes treated with white garlic extracts and inoculated with *M. phaseolina* gave highest inhibition percentages of 91.5 and 82.6% in water and ethanol garlic extracts, respectively, relative to the control plate at concentration of 250 ppm (Table 1). Similarly, Petri dishes treated with common pink garlic and inoculated with *M phaseolina* showed reduction of mycelial growth of 72.0 and 65.1% respectively on water and ethanol pink garlic extract after 7 days of inoculation. The least percentage inhibition of 27.7% was observed on *C. capsici* when treated with the common pink garlic while the white variety showed its least inhibition percentage of 34.45% on *C. destructivum* (Table 1). This *in-vitro* result on *M. phaseolina* was not significantly different at P=0.05 and compared favourably with conventional fungicide benomyl at 0.5 gai/kg (recommended concentration rate). Hence, only white garlic extract was tested in the *in-vivo* study.

There were general reductions in germination and plant stands of cowpea varieties when treated at 100% concentration of white garlic extract at 7 and 21 days after sowing (DAS) compare with 50% and untreated control. This is an indication of phytotoxicity of the extracts at 100% concentration on both cowpea varieties. However, cowpea variety TVx 3236 at 50% concentration of garlic extract gave complete seed germination (100%) when artificially inoculated with *M. phaseolina* and higher plant stand than cowpea variety IT84S-2246-4 at 50% concentration (Table 2). Seedling damping-off incidence, severity and control of both pathogens were greater at 50% garlic extract concentration at 7DAS in the greenhouse. However, the efficacy of the extracts reduced with days on cowpea variety IT84S-2246-4, such that by 21DAS control experiments gave higher cowpea stands than cowpea stands treated with 50% garlic extract concentration. On the other hand, germination, final stand and control of seedling damping –off caused by *C. capsici* was complete (100%) on cowpea variety TVx 3236 when treated with 50% garlic extract concentration. One hundred percent concentration was however not good for the control of damping –off diseases caused by the two pathogens and on either of the two cowpea varieties (Table 2).

DISCUSSION

Garlic extracts effectively inhibited the mycelial radial growth, conidial and sclerotial germination of the three pathogens *in vitro*. This observation is in agreement with the work of Sangoyomi (2004) who reported that aqueous extract of garlic effectively inhibited mycelia growth, conidia, pycnidia and sclerotial production of *Butryodiplodia theobromae, Aspergillus niger, Sclerotium rolfsii, Rhizoctonia solani,* and *Nattrassia mangifera,* fungal pathogens of yam in storage. Islam al. (2001) reported the control of C*olletotrichum* sp. and *M. phaseolina* in jute using garlic extract at ratio 1:2. Recent studies from Perelló et al. (2013); Baraka et al. (2011) also supported efficacy of garlic extract in control of wheat pathogens and/ or its oil extract at 100 and 500 ppm against root rot fungal pathogens of date palm. Allicin found in garlic are highly volatile (Freeman and Kodera, 1995) and readily membrane permeable. Slusarenko et al. (2008) described the constituents of damaged garlic tissues as volatile antimicrobial substance allicin (diallylthiosulphinate) and the substrate alliin (S-allyl-L-cysteine sulphoxide) which mixes with the enzyme alliin-lyase. Allicin also undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. This mode of action is suggested as the basis for its antimicrobial activities. In their studies, garlic juice was effective against some ranges of bacteria, fungal and oomycetes.

Water or ethanol garlic extract inhibited the growth of fungal pathogens in this study and it conforms to previous studies by Onyeagba et al. (2004); Tagoe et al. (2009).

This study also found that while both water and ethanol extracting solvents are effective solvent for extraction of antimicrobial constituent from garlic, garlic water extract gave higher control than its ethanol counterpart. Although

Table 2. Effect of concentrations of garlic extracts on germination and control of seedling damping-off of two cowpea varieties.

Pathogens	Garlic concentration	Cowpea varieties			
		TVx 3236		IT84S-2242-4	
		Germination %		Germination %	
M. phaseolina		**7DAS**	**21DAS**	**7DAS**	**21DAS**
	0	66.67	61.11	66.67	66.67
	50	100	55.56	77	16.67
	100	0	0	0	0
	Mean	55.56	38.89	47.89	27.78
	SE	29.40	19.51	24.13	20.03
C.capsici		**7DAS**	**21DAS**	**7DAS**	**21DAS**
	0	55.55	22.23	55.26	33.33
	50	100	100	100	0
	100	0	0	0	0
	Mean	51.83	40.74	18.46	11.11
	SE	28.93	30.32	18.42	11.11

Where DAS = Days after sowing;

not significantly different (P = 0.05), but the ease of extraction and reduced cost of producing potential bio-fungicides from garlic was demonstrated in this study. Studies on garlic extracts by Rasul et al. (2012) corroborated this finding. They reported that garlic water extract was superior and resulted in better extraction yield than ethanol water or methanol water garlic extracts. The fact that white garlic extract consistently proved more effective at inhibiting the mycelial growth of the three pathogens than the pink garlic, is an indication of variation in active constituents due to the existing garlic varieties. Most information on antifungal potential of garlic had not distinguished garlic potencies by their existing varieties. Climatic variation significantly affects color, shape and variety of garlic grown in a particular area (www.seeds.ca/proj/gcgc/). However, this study suggests that garlic varieties grown within the same climatic environment (tropical environment) exhibit antifungal variations on some cowpea pathogens due to their varieties. Germination of cowpea seedlings also varied at different concentrations and between the varieties tested. Permeability and tolerance level of cowpea variety to extracts may be responsible for such response. Permeability to water, oxygen and radicle protrusion properties of seed coat were positively correlated with seed coat color due to phenolic compound present in different species (Debeaujon et al., 2000). Zamin and Ajmal (2010), found that water uptake pattern of seed coat is dependent on exposure to saline or non-saline conditions as well as seed variety. Singh et al. (1980) also observed that neem oil extract inhibited the germination of *Cicer arietimum* gram seed at high concentration. As suggested by Ekpo (1999), pre-determination of extract concentration prior to application would bring out the expected benefits of natural fungicides.

Control of *M. phaseolina* on cowpea variety TVx3236 at 50% concentration showed gradual reduction from 100 to 55.56% at 7 and 21DAS, respectively, compared with 77.0 and 16.67% on cowpea variety IT84S-2246-4. This result suggests instability of garlic extract in controlling the pathogens with days. Rasul et al. (2012), reported decrease in pH as well as increase acidity of garlic water extract with time. Hence, application of garlic extract on seedlings along with seed treatment is suggested. According to Freeman and Kodera (1995) garlic extract easily loses its stability. Total control (100%) of *C. capsici* was observed on cowpea variety TVx3236 at 7 and 21 DAS. However, on cowpea variety IT84S-2246-4 control at 7DAS was 100% but there was no control at 21DAS. Adebitan et al. (1992) reported the resistance to *C. capsici* by cowpea variety TVx 3236 in the field. These studies therefore suggest that application of 50% garlic extracts concentration on the cowpea pathogens was more suitable as natural fungicides. It also showed that garlic extracts can be used to control the pathogens if applied at appropriate concentrations and can serve as cost reduction and environmentally friendly alternative to synthetic fungicide against seedling damping-off of cowpea caused by *M. phaseolina* and *C. capsici in-vivo.* It is important to note that high inoculum level employed under this study is not always obtained in nature. Therefore, the study may have under-estimated the potency of garlic extract under field condition. Area of further research will be to assess the effect of garlic extract under field conditions at different agro-ecological zones. Also, improvement on phytotoxicity by working on the volatility of garlic extract will enhance antimicrobial benefits of garlic extract on other cowpea varieties. It is believed that cheap and readily available natural fungicide can be obtained from this type of work.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

We are grateful to the International Institute of Tropical Agriculture (IITA) Ibadan Nigeria for financial support for this research work.

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