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Full Length Research Paper

Evaluation of antifungal efficacy of some plant extracts on *Curvularia lunata*, the causal organism of maize leaf spot

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The efficacy of leaf extracts of *Gliricidia sepium, Tithonia diversifolia, Phyllanthus amarus* and *Morinda lucida* were assessed *in vitro* to control *Curvularia lunata.* The extracts of the four plants suppressed the growth of *C. lunata in vitro*. All the extracts at 100% concentration significantly suppressed the growth of *C. lunata* (P ≤ 0.05). At all concentrations, *P. amarus* is most efficacious of all the plants extracts; this was followed by extract of *T. diversifolia* and *M. lucida*. Extract of *G. sepium* was the least effective of all the plant extracts against *C. lunata*. *P. amarus* is most efficient in the control of leaf spot of maize caused by *C. lunata* of all the four plant extracts used.

Key words: Botanicals, *Curvularia lunata*, extracts, leaf spot, maize.

INTRODUCTION

Maize, *Zea mays* L. is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production (IITA, 2007). Maize is high yielding, easy to process, readily digested and cheaper than other cereals. It is also a versatile crop, growing across a range of agro ecological zones. Every part of the maize plant has economical value: the grain, leaves, stalks, tassel and cob can all be used to produce a large variety of food and non-food products (IITA, 2007).

With this importance of maize, it is being plagued by an array of diseases which include the leaf spot of maize which is caused by *Curvularia lunata*. This disease is a very important seed and soil borne disease prevalent in the hot, humid maize areas. The disease produces small necrotic or chlorotic spot with a light colored halo; lesions are about 0.5 cm per spot when fully developed and this cause significant damage to maize up to 60% due to great loss of photosynthetic region of the crop.

Attempts have been made to develop maize cultivars that are resistant to leaf spot, and many other control measures have also been used to check this fungal disease. These include improved cultural practices on the farm and chemical control using fungicides and were then found to be effective against leaf spot when tested. But, most of these fungicides are not available to peasant farmers because most of the fungicides are expensive, require skilled labour and add to the cost of production while the yield obtained by their use may not be sufficient to justify cost of production. Also, most of these fungicides are toxic to humans and with the dwindling foreign exchange and prohibitive cost; most of the useful fungicides are usually out of reach of peasant farmers who are regarded as the producers of food in Nigeria (Anon, 1987). The pathogen on its own, also build up resistance to the fungicides and even when resistant varieties are planted in endemic areas.

These constraints have stimulated studies on the development of safer, cheaper, and effective control measures against leaf spot of maize and that can be recommended to all maize growers. This has brought about the introduction of the use of natural plant extracts in the control of this disease, which will have no side effect on the agro ecosystem, readily available and require little or no skill for its application. In order to combat pest and pathogen attacks, plants have over the years developed a number of protective mechanisms such as repellent pesticidal action. Aqueous extracts of some plants are known to have toxic properties, roots, leaves and other parts of plants contain chemicals which when present in sufficient concentration exerts toxic effect on the plant pathogens.

Gliricidia sepium is relatively free from insects and disease problems (Boa and Lenne, 1996). Tinctures made from the leaves of *G. sepium* were also found to inhibit the growth of various strains of *Neisseria gonorrhoea*

Table 1. Effect of different concentrations of 4 plant extracts on the growth diameter (cm) of *C. lunata.*

Concentrations (%)	48 h	96 h
0(control)	1.6d	2.7d
25	1.20c	1.83c
75	.95b	1.48b
100	0.77c	1.16a

Mean concentrations showed significant difference at P ≤ 0.05.

in *in vitro* test (Caceres et al., 1995). It was also reported that the presence of *G. sepium* in the fields reduces incidence of some fungal and insect attacks (Glover, 1989; Stewart, 1996). *Tithonia diversifolia* a bushy perennial weed is commonly found in the fields, (Akobundu and Agyakwa, 1987). The plant is used in the treatment of stomach pains, indigestion, sore throat and liver pains among the Luo tribe of Kenya (Kokwaro, 1976). Extracts of various parts of the plant have been reported to exhibit antimalaria (Madureira et al., 2002), antiproliferation (Gu et al., 2002), antidiarrhoeal (Tona et al., 1999), anti-amoebic and spasmolyic activities on humans (Tona et al., 1998). *Phyllanthus amarus* is a small, erect, annual weed that grows up to 30 – 40 cm in height and have small yellow flowers (Wikipedia, 2009). Researchers proved that extracts of *P. amarus* has antispasmodic properties (Santos et al., 2000) antimicrobial activity (Mazumder et al., 2006). *Morinda lucida* dried leaves extracts significantly suppressed the level of parasitemia after *Trypanosma brucei* infection in mice (Asuzu and Chineme, 1990). These plants have been reported to have effect on human infection; there is also the need to test their efficacy in plant disease control. The objective of this study was to get the most effective plant extract within farmers' reach to control *C. lunata* causing leaf spot of maize.

MATERIALS AND METHODS

Collection of plant samples and isolation of *C. lunata*

Infected samples of leaves ART/98/SW6 maize variety were collected from the field and brought to the laboratory, and cut into pieces. These were then surface sterilized for 1 min in 10% solution of sodium hypochlorite, rinsed in 5 changes of sterile distilled water and dried on sterile paper towel before inoculating on potato dextrose agar (PDA) in petri dishes and were later subcultured for pure culture.

The leaves of *M. lucida, T. diversifolia, G. sepium* and *P. amarus* were collected from the research field of Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria.

Extraction of the plant materials

The leaves of *M. lucida, T. diversifolia, G. sepium* and *P. amarus* were washed with tap water, air dried and then packed in envelope and kept in the oven at 80°C for 48 h, separately. The dried leaves were then blended into powder. Sterile distilled water of 100 ml was added to it and the suspension was heated over waterbath at 70°C for 20 min. The content was filtered using a piece of muslin cloth, and autoclaved for 10 min at 1.05 kg^{-2}cm. The plant extracts of 5 ml each were first poured into petri dishes. Then, molten PDA at 45 – 50°C was poured aseptically on the plant extract in the petri dishes and swirled round for even dispersion of the extract into the agar. The extracts were incorporated at different concentrations of 100%, 75, 25 and 0%. A 5 mm mycelium agar disc of *C. lunata* was released into the poisoned agar/ extracts incorporated into PDA with the agar diffusion method. The treatments were replicated three times, incubated at room temperature of 28 – 30°C and measurement of the mycelia growth extension of the fungus was taken at 48 h after incubation and at every 24 h of incubation. The experiment was set up in a completely randomized design (CRD); analysis of variance was carried out on the growth rate data by using SAS (1985).

RESULTS

The four botanicals (plant extracts) were separately incorporated into the growth medium of the target organism to determine the best botanical, appropriate concentration for application and its effectiveness on the pathogen. The mycelia extension of the organism was 1.13, 1.16 and 1.44 cm at 100, 75 and 25% plant extract incorporation of *T. diversifolia* into the growth medium. By the fourth day the mycelia growth of the pathogen in different concentration of *T. diversifolia* were 1.33, 1.63 and 1.70 cm respectively. This observation showed that 100% of plant extract incorporated into the growth medium was more effective in inhibiting the growth of *C. lunata* when compared to the 75 and 25% (Table 1).

At 48 h of incubation, the mycelia growth of *C.lunata* was 0.70, 1.14 and 1.56 cm at 100, 75 and 25% respectively of extract from *M. lucida*. The mycelia growths of the pathogen on the 4th day of these concentrations of the extract were 1.03, 1.57 and 2.54 cm. This observation showed that growth of the target organism on poisoned plates with *M. lucida* was inhibited compared with the growth on control plates which were not poisoned with extract of *M. lucida*. There was a significant difference in the growth of *C.lunata* on plate with *M. lucida* extracts. The 100% concentration was observed to be the most effective (Table 1).

G. sepium extract was incorporated in agar at different concentrations of 100, 75 and 25%, after 48 h of incubation; the mycelia extension of the organism was 0.68, 0.87 and 1.21 cm on different concentrations respectively. By the 4th day, the mycelia extension was 1.37, 2.27 and 2.44 cm respectively. These observations showed that there was inhibition in growth by the 4th day as the mycelia growth reduced compared with the growth on control plates which were not poisoned with extract of *G. Sepium*. Among the concentrations, 100% was observed to be most effective (Table 1). There was growth inhibition of *C. lunata* by *P. amarus* at 48 h of incubation of 100, 75 and 25% of the plant extract incorporated into the growth medium; the

Table 2. Effect of different plant extracts at 75% on the mycelia growth (cm) of *C. lunata.*

Treatment	Time	
	48 h	96 h
P. amarus	0.60a	0.67a
T. diversifolia	1.24c	1.54b
M. lucida	1.13c	1.71b
G. sepium	0.92b	2.26c
LSD (0.05)	2.70	4.60

Table 3. Inhibitory effects (cm) of plant extracts on *C. lunata*.

Plant	Concentration (%)	48 h	96 h
P. amarus	100	0.66	0.64
	75	0.64	0.74
	25	0.60	0.77
T. diversifolia	100	1.13	1.33
	75	1.16	1.63
	25	1.44	1.70
M. lucida	100	0.70	1.03
	75	1.14	1.57
	25	1.56	2.54
G. sepium	100	0.68	1.37
	75	0.87	2.27
	25	1.21	2.44
	0	3.52	5.10

growth was 0.58, 0.64 and 0.60 cm respectively. By the 4th day, mycelia extension of the target organism was 0.64, 0.74 and 0.66 cm. There was no significant difference among the different concentrations of the plant extracts two days after inoculation but significant difference was noted at fourth day of incubation (Table 2).

Extracts of *P. amarus* had the highest inhibitory effect in all the plant extracts used (Table 2). There was increase in inhibitory effect on the growth of the target organism as the concentration of the plant extract increased.

DISCUSSION

Plants in their natural state possess a relative stable biological balance with microbes on their surface. An alien organism introduced into an area in which it has no natural enemies may increase in number to such an extent that the resident population is unable to redress this imbalance. Almost any process occurring naturally or done artificially which affect the growth of populations of organism in such a way that the natural biological balance is restored or not affected, is what the use of these plant extracts aimed to achieve in this study. There was significant difference at $P \leq 0.05$ in the growth of the target organism at the 4th day compared with the previous days and there are possibilities for further inhibition of growth of the pathogen by the extracts as the incubation period increases.

The result suggests that the extract of these plants have inhibitory effects on the growth of *C. lunata* (Table 3). *G. sepium* was able to inhibit the pathogen growth though as other plant extracts probably due to the tannins present in the plant (Jackson et al., 1994) or other compounds such as aformosin, medicarpin or some lsoflavins (Herath et al., 1998). Some of the bioactive compounds that have been isolated from the leaves include sesquiterpenes, saponins and alkaloids (Tona et al., 2000) and made *T. diversifolia* active in inhibiting mycelia growth of *C. lunata*. The hydrolysable tannin geraniin of *P. amarus* was seven times more potent as a pain reliever (Miguel et al., 1996). Methanol extracts of *M. lucida* shows analgesic, antipyretic effects and potentiated phenobarbitone sleeping time (Awe et al., 1998).

The most effective among the plant extracts was the extract of *P. amarus,* followed by extract of *T. diversifolia*. Extract of *M. lucida* also had inhibitory effect but not as

much as the extracts of *P. amarus* and *T. diversifolia*. Extract of *G. sepium* was the least effective on *C. lunata*. The potentials of these plants for pathogen control have not been fully realized largely because the experiment was performed *in vitro.* However, their effectiveness in field condition could be of a potential advantage as it will help to determine the *in vivo* inhibitory effect of the botanicals.

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