

Antimicrobial effects of botanicals and biocontrol agents against *Phaeoisariopsis personata*, the causal agent of Tikka disease of groundnut

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Abstract. Ndifon EM. 2023. Antimicrobial effects of botanicals and biocontrol agents against *Phaeoisariopsis personata*, the causal agent of Tikka disease of groundnut. *Asian J Agric* 7: 71-75. Groundnut is highly susceptible to various pathogenic diseases, the most important of these is tikka. The aim of this study was to control the infection of groundnut caused by *Phaeoisariopsis personata* (Berk. & M.A.Curtis) Arx using two experiments. Each trial was set up using a completely randomized design, and each treatment was replicated thrice. Results show that *Eucalyptus globulus* Labill. resinous gum (at 50% and 100% concentrations) and *Terminalia catappa* L. resinous gum (at 50% concentration) were more effective in the inhibition of *P. personata*, followed by *Anacardium officinale* Pritz. resinous gum and *T. catappa* resinous gum (at 100% concentration). All the plant extracts significantly ($P \leq 0.05$) inhibited the pathogen. The percentage inhibition of *P. personata* by plant extracts ranged between 7.9-100%. All the isolates of *Trichoderma* were able to inhibit *P. personata* effectively, but the percentage inhibition of mycelial growth of *P. personata* reduced gradually with time. The percentage inhibition of *P. personata* by *Trichoderma* species ranged between 24-100%. The best biocontrol treatments were *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB, and *T. harzianum* isolate ZXMZ. Using these biocontrol and botanical measures to manage the tikka leaf spot of groundnut is highly recommended.

Keywords: Leaf spots, peanuts, plant extract, *Trichoderma* species, yield loss

INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L. in the plant family Fabaceae) is a major oil crop rich in vitamins, protein, grease, oil, and fiber (Settaluri et al. 2012; Karmini et al. 2017). Peanuts are consumed worldwide in various forms, mostly in traditional cuisine. In addition, peanuts are widely used to produce peanut butter, confectionaries, roasted peanuts, snack products, soups, and desserts. Groundnut is essential in reducing malnutrition among the population in many African countries (Guimon and Guimon 2012).

Groundnut is cultivated to produce cooking oil, animal feed, groundnut flour, and for boiling/roasting as a snack (Patil and Pillai 2010; Awurum and Uwajimgba 2013). Groundnut is ranked sixth among the most important oil-seed crops globally (Koita et al. 2017). Groundnut seeds are rich in edible oil (50%), protein (25-30%), carbohydrate (20%), fiber, and ash (5%) (Awurum and Uwajimgba 2013). Groundnut is cultivated globally (including in Nigeria). Nigeria is among the first three groundnut-producing countries in the world. Groundnuts are cultivated throughout Africa, Asia, and the Americas, but the crop suffers severe yield loss due to diseases like Tikka disease. It is cultivated on a large scale in Abakaliki, Nigeria, despite being prone to leaf spot diseases. Like any other plant, groundnut is susceptible to more than 55 pathogens, including fungi, bacteria, nematodes, and viruses. Fungi are the causal agents of Tikka disease (Patil and Pillai 2010; Awurum and Uwajimgba 2013; Sangoyomi and Alabi 2016; Damicone 2017). Two fungi induce tikka leaf spot

disease, namely groundnut early leaf spot disease otherwise called *Cercospora* leaf spots or brown leaf spots or peanut cercosporiosis (induced by *Cercospora arachidicola* Hori. (Perfect stage - *Mycosphaerella arachidicola* W.A. Jenkins), and late leaf spot disease (caused by *Phaeoisariopsis personata* (Berk. & M.A.Curtis) Arx (Perfect stage - *Mycosphaerella berkeleyi* W.A. Jenkins).

The control of Tikka disease using biocontrol agents is in its rudimentary stage. Culture-filtrates of *Penicillium islandicum* Sopp. (50% concentration, applied at 70 DAS (i.e., days after sowing) proved potent against some pathogenic fungi of groundnut. It has been confirmed that groundnut seeds dressed with *Pseudomonas fluorescens* Rhodes formulation exhibited less disease severity after inoculation with late leaf spots. Moreover, *Trichoderma viride* Pers. (5% concentration) and *Verticillium lecanii* Zimm (5% concentration) were applied, significantly reducing the severity of leaf spot disease (Deyd and Dhutraj 2014).

Pal and Gardener (2006) emphasized that further development and effective adoption of available biocontrol agents require a greater understanding of the complex interactions among plants, people, and the environment. In addition, Yazdani et al. (2011) reiterated that using botanical fungicides (which are largely nonphytotoxic, nonsystemic, and easily biodegradable) can be an effective replacement for synthetic fungicides.

Studying the antimicrobial action of plant extracts and biocontrol fungi isolates is the first step to optimizing the use of these agents to protect groundnuts against Tikka disease and improve groundnut production. Therefore, the

aim of the present study was (i) to evaluate the antimicrobial activity of plant extracts and (ii) to assess the antimicrobial activity of some *Trichoderma* isolates against Tikka diseases of groundnut. The control of Tikka diseases of groundnut can be a determining factor in the production of groundnut.

MATERIALS AND METHODS

Study site

This study was conducted in the semi-humid tropics at the Faculty of Agriculture Laboratory complex, Alex Ekwueme Federal University Ndufu-Alike, Abakaliki, Nigeria (6.069°N by 8.199°E). Abakaliki experiences rainfall for more than nine months in a year. Therefore, the average relative humidity is usually high throughout the year. The mean relative humidity in the area remains above 70% for nine months per annum; thus, pathogenic propagules can easily survive in the environment. Abakaliki is located in the derived savannah ecological zone of Nigeria. It has well-drained iron-rich loamy soils.

Isolation and identification of the fungi

The infected groundnut leaves used for this research were obtained from the West Cameroons (05.120 - 06.220N by 09.180-10.220E). The groundnut leaves were collected from mature groundnut plants. *Trichoderma* isolates were obtained from groundnut seeds, mushrooms, crop seeds (i.e., maize, cowpea, pigeon pea, Bambara groundnuts, guava, mango, pawpaw, garden eggplant, orange, lemon, lime, eggplant, cacao, kidney beans, carrot, onions, sorghum, rice, millet, palm kernel nuts, roselle, date palm, cotton seeds, fig, lima bean, tomato, kola nut, and bitter kola). They were also obtained from farmland soils in the West Cameroons, as well as from northern (10.517N by 07.433E), north-central (10.376N by 07.709E), and south-eastern (09. 519N x 08.403E) Nigeria. The crop seeds were bought from markets and farmers/farms. The seeds were in a dehydrated state, and each seed was packaged in a separate envelope until it was processed later.

The fungi (*P. personata* and *Trichoderma* Pers.: Fr. spp.) were cultured on Acetate Differential Agar® enriched with dextrose and autoclaved at 120°C and 15 psi for 15 minutes according to the manufacturer's (Difco; USA) instructions. The isolated fungi were sub-cultured to obtain pure cultures (Ndifon 2022a) and identified with the aid of literature on fungi morphology (Narayanasamy 2011; Subrahmanyam et al. 2012; Fischbach and Dunning III 2015; Aglave 2019).

Experiment 1: Evaluation of the effects of plant extracts bio-assayed against tikka leaf spot disease

This poison food technique experiment was laid out in the laboratory using a Completely Randomized Design (CRD) with seven treatments, and each treatment was replicated three times. The treatment set consisted of the control, *Anacardium officinale* Pritz. resinous gum (50% and 100%), *Terminalia catappa* L. resinous gum (50% and

100%), and *Eucalyptus globulus* Labill. resinous gum at 50% and 100% concentrations.

The plant exudates (i.e., *E. globulus* resinous gum, *T. catappa* resinous gum, and *A. officinale* resinous gum) were utilized (at the rate of 166.7 g resinous gum per L of distilled water) to make 100% concentration of each plant extract. The plant extracts were strained through double-layered muslin clothes and filtered through Whatman's No. 1 filter paper. Each petri dish contained 15 mL of Acetate Differential Agar enriched with dextrose and the plant extract specified in the randomization/layout. The control received no plant extract and was inoculated in the same way with the Tikka disease pathogen (i.e., with a 2-mm disc of culture) as the other treated plots.

Experiment 2: Evaluation of the effects of *Trichoderma* isolates against tikka leaf-spot disease pathogen

This in vitro dual-culture experiment was laid out using CRD, and each treatment was replicated three times. The treatment set consisted of nine *Trichoderma* isolates (i.e., *T. harzianum* isolate BGMZA, *T. hamatum* (Bon.) Bain. isolate ZXPB, *T. harzianum* isolate ZXMZ, *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* Pers.: Fr. isolate AIBK, *T. harzianum* isolate AIBN, *T. harzianum* isolate ZXGV, and *T. virens* (J. Miller, Giddens & Foster) von Arx, Beih. isolate BGMZ2). The control was inoculated with *P. personata* only.

Each petri dish contained 15 mL of Acetate Differential Agar enriched with dextrose, which was autoclaved at 120°C and 15 psi for 15 minutes according to the manufacturer's instructions. Discs from the cultures of pathogen and *Trichoderma* isolates were cut using a 2-mm cork borer and placed at the edge of the Petri dishes. The control received no *Trichoderma* isolate and was inoculated the same way as other treated plots.

Data collection for both trials

The radius of the fungal colony was measured using a transparent ruler at 24-hour intervals starting from day 1 through day 7. The percentage inhibition was calculated using the following equation (Ndifon 2022b):

$$PI\% = ((C - T)/C) \times 100$$

Where:

PI : % inhibition of growth of the fungus

C : Perpendicular* radius of the fungal colony in the control plate

T : Perpendicular radius of the fungal colony in treated plate

*Perpendicular refers to the 'right angle' through the center of the Petri dish.

Data analysis

The data were subjected to an Analysis of Variance (ANOVA) procedure, and the means were separated using Duncan's Multiple Range Test (DMRT) at 5% probability level (as obtainable with Genstat® Discovery 2nd Edition statistical package). The descriptive statistics were assessed using IBM Statistical Package for Social Sciences (SPSS) version 25 and Microsoft Excel 365 procedure).

RESULTS AND DISCUSSION

The trend of percentage inhibition of radial growth of *P. personata* due to the application of plant extracts in vitro is presented in Table 1. The result shows that 50% and 100% concentrations of *E. globulus* resinous gum plant extract and 100% concentration of *T. catappa* resinous gum plant extract showed complete inhibition of *P. personata*. A 50% concentration of *A. officinale* resinous gum plant extract was the least effective treatment for managing *P. personata*. The percentage inhibition of *P. personata* by the plant extracts ranged between 7.9-100% inhibition. The results reveal that during the early stage of the trial (at 24 hours after inoculation (i.e., HAI)), a significantly different ($P \leq 0.05$) percentage inhibition existed between the treated experimental units and the control. *E. globulus* resinous gum plant extract at 100% concentration exhibited the highest inhibitory potential against the pathogenic species, followed by *E. globulus* resinous gum plant extract at 50% concentration.

This trend of inhibition was maintained (as at at previous data collection intervals) at 72 HAI, except that *A. officinale* resinous gum plant extract (at 50% concentration) showed dismal performance compared to the control. This treatment, however, performed significantly better than the control at 120 HAI. A clear pattern of inhibition was observed at 120 HAI, whereby *E. globulus* resinous gum plant extract treatments (at 50% and 100% concentrations) topped the charts, followed by *T. catappa* resinous gum plant extract at 50% concentration, *A. officinale* resinous gum plant extract (at 100% concentration) and *T. catappa* resinous gum plant extract (at 100% concentration). All the treated plots significantly inhibited the pathogen during the trial.

The percentage inhibition of mycelial growth of *P. personata* by applying biocontrol agents in vitro is presented in Table 2. The results show that at 24 HAI, all the isolates performed above average except for *T. harzianum* isolate AIBN. All the biocontrol agents effectively inhibited the radial growth of *P. personata* compared to the mean inhibition pattern. However, the percentage inhibition of the radial growth of *P. personata* reduced with time. The percentage inhibition of *P. personata* by *Trichoderma* species ranged between 24-100% inhibition, with the mean inhibition being 40%.

Therefore, to verify the probability of these observed differences being specious among the biocontrol treatments, a

representative extract of some intervals of the data was subjected to the ANOVA procedure, and the means were separated using the new Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) as publicized in Table 2. The results of statistical analysis revealed that during the mid-way stage of the trial (72 HAI), a significantly different ($P \leq 0.05$) percentage inhibition existed between the treated experimental units and the control. In addition, the results from the experimental unit - *T. harzianum* isolate AIBN was significantly different ($P \leq 0.05$) compared to the other treated experimental units.

At 120 HAI, all the treated experimental units showed significantly different ($P \leq 0.05$) percentage inhibition of the radial growth of the pathogen compared to the control. From the results of the last data collected at 168 HAI, a clearer percentage inhibition pattern was observed among the treatments. All the treated experimental units inhibited the growth of *P. personata* mycelia significantly compared to the control. The best treatments were *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB and *T. harzianum* isolate ZXMZ followed by *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* isolate AIBK, *T. harzianum* isolate AIBN, and finally *T. harzianum* isolate ZXGV.

Discussion

In this study, all the plant extracts successfully inhibited the radial growth of *P. personata*. Ndifon and Lum (2021) reported that the leaves of *E. globulus* and four other botanicals (at 50 and 100% concentrations) inhibited the growth of *Aspergillus niger* van Tiegh. on white yams significantly compared to the negative control, and the level of inhibition was higher at the 100% concentration than at the 50% concentration. Apet et al. (2015) conveyed that *Ceratocystis paradoxa* (Dade) C. Moreau was effectively controlled by all the botanicals applied as follows: *Allium sativum* L. (63.9% inhibition), followed by *Zingiber officinale* Roscoe (61.5% concentration), and *Azadirachta indica* A.Juss. (59.8% concentration). These findings are in agreement with those of the current study. Moreover, Agbenin et al. (2010) publicized that neem seed powder significantly reduced the performance of *Fusarium* Syn. & Hans. and *Meloidogyne* Goeldi spp. in both the screen house and the field. Thus, plant extracts have a wide spectrum of microbes that they can control simultaneously.

Table 1. The effects of plant extracts on the radial growth of *Phaeoisariopsis personata*

Treatments	Percentage Inhibition (%)					Mean of Colony Radius (cm)		
	24h	48h	72h	96h	120h	24 h	72 h	120 h
<i>Eucalyptus globulus</i> resinous gum 100% concentration	100.0	100.0	95.2	96.2	96.7	0.0a	0.1a	0.1a
<i>Anacardium officinale</i> resinous gum 100% concentration	85.2	50.0	46.0	28.2	32.2	0.1a	1.1c	2.0c
<i>Terminalia catappa</i> resinous gum 100% concentration	80.3	47.5	42.9	29.5	27.8	0.1a	1.2c	2.2c
<i>Eucalyptus globulus</i> resinous gum 50% concentration	100.0	77.5	79.4	83.3	85.6	0.0a	0.4ab	0.4b
<i>Anacardium officinale</i> resinous gum 50% concentration	45.9	12.5	7.9	29.5	23.3	0.4b	1.9d	2.3d
<i>Terminalia catappa</i> resinous gum 50% concentration	100.0	57.5	52.4	33.3	33.3	0.0a	1.0bc	2.0c
Control**	0.0	0.0	0.0	0.0	0.0	0.7c	2.1d	3.0e
Mean	85.2	57.5	54.0	50.0	49.8	0.2	1.1	1.7
SED	-	-	-	-	-	0.06	0.28	0.10
FLSD ($P \leq 0.05$)	-	-	-	-	-	0.13	0.62	0.22

Note: **Percentage inhibition by control is zero based on the equation. Treatment means followed by the same letter(s) in a column are statistically similar using DMRT ($P \leq 0.05$)

Table 2. The effects of *Trichoderma* isolates on the radial growth of *Phaeoisariopsis personata*

Treatments	Percentage inhibition (%) of the radial growth of the fungus								Ranking of the means of radial growth of fungus		
	24 h	48 h	72 h	96 h	120 h	144 h	168 h	Area (%)	72 h	120 h	168 h
<i>T. harzianum</i> isolate AIBN	23.8	52.5	56.3	59.5	51.1	50.5	46.7	60.0	0.5b	1.0a	2.1ab
<i>T. viride</i> isolate AIBK	100.0	70.0	62.5	58.2	54.4	49.5	51.6	43.3	0.0a	1.1a	2.0ab
<i>T. virens</i> isolate BGMZ2	90.5	62.5	65.6	58.2	52.2	46.8	41.8	43.3	0.1a	1.1a	1.2a
<i>T. harzianum</i> isolate BGMZ4	85.7	62.5	65.6	62.0	55.6	55.0	57.4	43.3	0.1a	1.0a	1.7a
<i>T. hamatum</i> isolate ZXPB	100.0	80.0	70.3	58.2	50.0	52.3	57.4	40.0	0.0a	1.1a	1.7a
<i>T. harzianum</i> isolate ZXMZ	90.5	62.5	62.5	58.2	54.4	54.1	56.6	36.7	0.1a	1.1a	1.8a
<i>T. harzianum</i> isolate ZXGV	90.5	62.5	59.4	51.9	50.0	45.9	42.6	50.0	0.1a	1.3a	2.3b
<i>T. harzianum</i> isolate BGMPP	81.0	57.5	60.9	54.4	50.0	45.9	50.8	46.7	0.1a	1.2a	2.0ab
<i>T. harzianum</i> isolate BGMZ3	87.3	60.8	60.9	54.9	51.5	48.6	50.0	44.4	0.1a	1.2a	1.8ab
Control **	-	-	-	-	-	-	-	-	0.7c	2.6b	4.1c
Mean	83.2	63.4	62.7	57.3	52.1	49.8	50.5	45.3	0.2	1.1	1.7
SED	-	-	-	-	-	-	-	-	0.1	0.2	0.2
FLSD ($P \leq 0.05$)	-	-	-	-	-	-	-	-	0.2	0.3	0.5

Note: **Percentage inhibition by control is zero based on the equation. Treatment means followed by the same letter(s) in a column are statistically similar using DMRT ($P \leq 0.05$)

In this present study, all the *Trichoderma* isolates could effectively inhibit the radial growth of *P. personata*. The performance of the biocontrol agents revealed that their performance was isolate-dependent, and the best isolates were *T. virens* and some *T. harzianum* isolates, while *T. viride* isolate was the second-best biocontrol agent. Ndifon (2022a) affirmed these results with the findings that biocontrol agents (*Trichoderma* and *Cladosporium* Link Ex Fries spp.) significantly inhibited the radial growth of *Globisporangium ultimum* by 10-90% inhibition.

Verna et al. (2007) explained that *Trichoderma* spp. are antagonistic fungal agents usable against several pests and as plant growth enhancers. *Trichoderma* species exhibit faster metabolic rates, produce antimicrobial metabolites, carry out mycoparasitism, spatial and nutrient competition, antibiosis (by enzymes and secondary metabolites), and induction of plant defense system. These biocontrol agents can serve various purposes. That brings to mind the precaution requiring that we study these organisms more before utilizing them in new environments.

Kumar et al. (2014) reported that *T. harzianum* triggered maximum growth inhibition of *Sclerotium rolfsii* (Curzi) C.C. Tu & Kimbr) Sacc., *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk, and *Sclerotinia sclerotiorum* (Lib.) de Bary (at 96 HAI: to the tone of 57.0%, 58.4%, and 64.8% inhibition respectively). Apet et al. (2015) reported that *C. paradoxa* was effectively controlled by *T. viride* (77.0% inhibition), followed by *T. harzianum* (70.7% inhibition) and *T. hamatum* (69.4% inhibition). These results are very encouraging because of the high percentage inhibition of fungal growth, and many species of fungi have biocontrol potentials.

Basumatary et al. (2015) perceived that the highest inhibition of the growth of *S. rolfsii* was caused by *T. harzianum* (77.4%), followed by *T. viride* (76.5%). Lower rates of inhibition were obtained when *Aspergillus niger* (30.5% inhibition), *Penicillium* sp. (29.1% inhibition), and *Curvularia* sp. (13.6% inhibition) were applied. Additionally, Gajera and Vakharia (2012) showed that 12

isolates of *Trichoderma* (i.e., six of *T. harzianum*, five of *T. viride*, and one of *T. virens*) effectively reduced the incidence of collar rot disease of groundnut caused by *A. niger*. The *T. viride* showed the highest inhibition of *A. niger* (86.2%), followed by *T. harzianum* isolate 2J (80.4%) 6 days after inoculation. These results are fully in accord with the current findings presented herein.

Using a combination of different methods of pathogen control has been attempted, and the combination treatments were effective. For example, Srinivas et al. (1997) sprayed mancozeb+carbendazim at 50 Days After Sowing (DAS), followed by spraying of *Calotropis procera* (Aiton) W.T. Aiton leaf extract at 70 DAS and observed a highly significant reduction in the leaf spot disease.

Deyd and Dhutraj (2014) reported that applying Bavistin (i.e., BAU-bio-fungicide) followed by applying neem leaf extract + K_2O significantly reduced the severity of leaf spot disease. Hasan et al. (2014) expounded that neem leaf (*A. indica*), debdaru leaf (*Polyalthia longifolia* (Sonn.) Thwaites.), *datura* leaf (*Datura metel* L.), and BAU-bio-fungicide (Bavistin) foliar spray significantly controlled leaf spot disease compared to the control in the field.

This study scrutinized the antimicrobial effect of botanicals and biocontrol agents against the tikka leaf spot disease of groundnut. Two experiments were set up to determine if the plant extracts or biocontrol agents can suppress the growth of *P. personata* on groundnut. It can be concluded from the present study that all the plant extracts from *E. globulus* resinous gum, *A. officinale* resinous gum, and *T. catappa* resinous gum effectively inhibited the growth of *P. personata* in vitro. Furthermore, the *Trichoderma* species trial (i.e., as a biocontrol agent) revealed all *Trichoderma* isolates: *T. virens* isolate BGMZ2, *T. harzianum* isolate BGMZ4, *T. hamatum* isolate ZXPB, *T. harzianum* isolate ZXMZ, *T. harzianum* isolate BGMPP, *T. harzianum* isolate BGMZ3, *T. viride* isolate AIBK, *T. harzianum* isolate AIBN, and *T. harzianum* isolate ZXGV, they have effectively controlled the tikka leaf spot pathogen.

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