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Research article

Effect of Different Application methods of *Pseudomonas fluorescens* on growth of Groundnut (*Arachis hypogaea* l.) Infected with *Phaeoisariopsis personata*

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Abstract
The role of groundnut in improving food security and farmers' income in the Nigerian savanna cannot be over emphasized. Laboratory and pot experiment were conducted in the laboratory and screenhouse of the Department of Plant Biology, Bayero University, Kano in 2021 to study the effect of different application methods of *Pseudomonas fluorescens* on growth of groundnut (*Arachis hypogaea* L.) infected with *Phaeoisariopsis personata* causing late leaf spot of groundnut. Dual culture assay was used in the laboratory and pot experiment was conducted in screen house. Three groundnut varieties viz; SAMNUT 24, SAMNUT22 and Jar gyada. The treatments consisted of three different application methods of *P. fluorescens* (seed, foliar and combined seed and foliar) and controls (standard and negative control). Groundnut plants were artificially infected with 5×10^5 conidia per ml of *Phaeoisariopsis personata* and treated using the abovementioned methods. The experiment was arranged in a Complete Randomized Design (CRD) and all treatments and controls were replicated four times. Parameters measured included plant height, number of leaves and number of branches. Results of the study revealed that in dual culture experiment *P. fluorescens* inhibited the growth of *C. personatum* (64.70 and 70.58%). In pot study, SAMNUT 22 recorded the highest plant height (43.20) and the least was recorded by Jar gyada (36.95) while SAMNUT 24 recorded the highest number of leaves (136.8) and branches (34.25) and the least was recorded by jar gyada. Combined seed and foliar treatments recorded the highest value (37.75) in terms of number of branches. Therefore, it can be concluded that use of seed application followed by foliar spray of *Pseudomonas* enhance growth of *Phaeoisariopsis personata* of some groundnut varieties significantly.

Keywords: Application methods, *Pseudomonas fluorescens*, growth and *Phaeoisariopsis personata.*

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) originated from South America (Krapovickas, 2017) is an important food and cash crop in many countries worldwide and it contribute significantly to food security and alleviate poverty (Tan *et al*., 2020) in some developing countries like Nigeria. Asia alone produces 17.9- million tons (70% of global production). Africa produces another 20%with about 60% of Africa's production comes from Western Africa (FAO, 2006). Nigeria is considered among the main producing countries beside China, India, Sudan, United States of America and Indonesia (Mondal *et al*., 2006).

Moreover, the crop belongs to the family Fabaceae is the 13th most important food crop of the world and the world's 4th most important source of edible oil and 3rd most important source of vegetable protein (Taru *et al*.,2010). Nearly 75 to 80 % of world's groundnut is grown by resource poor smallholder farmers in developing countries who routinely obtain yields of 500 to 800 kg ha^{-1} as opposed to the potential yields of >2.5 t ha^{-1} (Idem and Showemimo, 2005). Low yields are mainly due to numerous diseases caused by fungi, viruses, bacteria and nematodes (Arogundade *et al*., 2010; Aliyu *et al*., 2011; Osei *et al*., 2013). Though Nigeria is the largest producer of

groundnut in Africa, fungal diseases remain a serious challenge to producers of the crops (Ncube and Maphosa,2020). Late leaf spot induced by *Phaeisariopsis personata* (Von Arx) was reported as the most prevalent and economically important fungal disease of groundnut worldwide that results to more than 50 % yield loss (Phat *et al*., 2019).

Although this disease can be controlled by fungicides, the use of fungicides increases environmental pollution and causes damage to the ecosystem. Moreover, frequent application of fungicides may lead to the development of fungicide resistance by the target organism (Azevedo *et al*., 2015).

Pseudomonas fluorescens encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium. As its name implies, it secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability. It is an obligate aerobe, except for some strains that can utilize NO_3 as an electron acceptor in place of O_2. It is motile by means of multiple polar flagella (Ganeshan and Kumar, 2006).

Three possible mechanisms have been suggested to explain the beneficial effects of PGPRs in enhancing production. They are competition for substrate and niche exclusion and production of siderophores and antibiotics. Fluorescent pseudomonads mop up nutrients in the rhizosphere because of their versatility in growth and nutrient absorption (Prasad *et al*., 2015). One of the major constraints facing the productivity and availability of healthy groundnut produce worldwide are the losses and spoilage caused by Fungi, bacteria, viruses, insects, nematodes and parasitic weeds. In fact, the threat to this crop from fungi species which produce secondary metabolites has now reached a level that outstrips that posed by other biotic and abiotic factor (Saurabh *et al*., 2014). These fungi continue to represent a major human health risk throughout the world and particularly in the humid tropics being major spoilage agents of food crops (Ashiq, 2015).

Several attempts were made to achieve biological control of groundnut diseases using biocontrol agents such as fungi and bacteria. Most of these studies were confined to greenhouse environments and a few have reached the point of commercial application. Although progress has been made in the experimental biological control of groundnut diseases, the mechanisms involved are yet to be fully understood. There is great potential for control of major groundnut diseases using growth promoting bacterial strains, thereby cutting the cost of agrochemicals applied and consequently, the yield loss (Dutta *et al*., 2011).

For the management of groundnut leaf spot, researchers mainly concentrates only on fungicides, which yielded success only to a limited extent, but encountering with undesirable problems (Munir, 2020).

Large scale utilization of chemical fungicides resulted in deterioration of soil fertility, residual toxicity, development of resistance by the pathogen and loss in biodiversity. To alleviate all these ill effects, environmentally safe, long lasting and ecofriendly, non-chemical means are in need for effective plant disease management since groundnut leaf spot disease has been reported tocaused severe damage on many crops especially groundnut in Nigeria, and has led to great reduction in yield. This study determine the effectiveness of different application methods of *Pseudomonas fluorescence* on the growth of groundnut infected with *Phaeoisariopsis personata*.

MATERIALS AND METHODS

Laboratory and pot experiment were conducted in the laboratory and screenhouse of the Department of Plant Biology, Bayero University, Kano in 2021.

Preparation of Bacterial Suspension

Phaeoisariopsis personata was isolated from leaf spot-infected groundnut leaves in accordance with the procedure of Meena *et al.,* (2002). The infected leaves were washed in running tap water for 5 min, surface sterilize in 0.53% (v/v) sodium hypochlorite for 1 min and then rinsed in sterile distilled water six times. Conidia were removed under aseptic conditions and placed on potato dextrose agar (PDA) and identification was done base on macroscopic and microscopic features. Conidial suspension was obtained from 3-weeks old cultures by flooding Petri plates with sterile distilled water and scraping them with a glass rod. Mycelial fragments was removed by passing the suspension through two layers of sterile muslin cloth.

A fungal inoculum was prepared from fourteen days old culture of *C. Personatum*. Culture plates were flooded with sterile distilled water (SDW) of ten mill (10ml) and the conidia were scraped using sterilized spatula. The conidial suspension was filtered through muslin cheese cloth and counted with a Hemocytometer. The concentration was adjusted to 5×10^5 conidia per ml (NurAinizati and Abdullahi, 2008).Isolation of *Pseudomonas fluorescence* was done by serially diluting the soil samples and a dilution of 10^3 was used as stock for the isolation of the organisms. Using a micropipette, 0.1 ml of the sample was drawn and plated onto already prepared Nutrient Agar (NA) and incubated at 27^0C in an incubator. Morphological characteristics of the colonies was used to identify the target organism. Colonies observed that resembled Pseudomonas spp. based on color and shape of colony was sub-cultured onto solidified nutrient agar (NA) which later was observed under microscope (Yakubu *et al*., 2019). The bacterial suspension was standardized by comparing the opacity with Macfaland tube to obtain a concentration of 1×10^6 cfu/

Biochemical characterization of *Pseudomonas fluorescens*

Starch hydrolysis

Filter paper was dipped in a day old culture suspension and was placed on Petri dishes containing starch agar medium and incubated for two days. The plates were than flooded with one per cent iodine solution. A colorless halo around the growth and blue color in the rest of the plates showed utilization of starch by the microorganism (Nepali *et al*., 2020).

Catalase Test

Capillary tubes (1mm outer isolated by 67mm in length) were placed in a 50-ml beaker containing about 10 ml of 3% H_2O_2. A positive result is the rapid evolution of oxygen by bubbling. These phenomena is due to the breakdown of hydrogen peroxide H_2O_2 in to water and oxygen (Nepali *et al*., 2020).

Oxidase Test

Saturate whatman number one (no.1) filter paper with 1% aqueous solution of Dimethyl-phenylene-diamine oxalate. Development of red colour within a 10seconds indicates a positive reaction (Nepali *et al*., 2020).

Gelatin Liquefaction

Filter paper discs were dipped in a day old culture suspension and were placed on a petridishes containing gelatin nutrient agar medium. The petri dishes were incubated at 30^0C for two days and then flooded with 12.5% HgCl solution. The development of yellow halo around the growth indicates utilization of gelatin (Nepali *et al*., 2020).

Fluorescent Pigmentation

The test tubes containing nutrient agar medium were inoculated with isolate of *Pseudomonas fluorescens* incubated for five days and observed under UV light. Yellowish green fluorescent pigment indicated positive result (Nepali *et al*., 2020).

Collection of Groundnut seed

Three varieties of groundnut (*Arachis hypogaea*) seeds (SAMNUT 22, SAMNUT 24 and Jar gyada) were obtained from International Institute for Semi-arid Tropics (ICRISAT) and Department of Agronomy, Bayero University Kano.

Dual Culture Experiment

Assay was performed in petri dishes? containing PDA without antibiotics. A 5mm diameter block of the *C. personatum* culture was placed at a distance of 1cm from the edge of the petridishes containing potato dextrose agar (PDA) culture medium. These were Incubated at 25°C for 48hour. After this, using a sterile loop, half of the petri dishes were inoculated with the suspension of bacteria. In the control treatment, sterile distilled water was used instead of bacterial suspension. Inhibition rates were evaluated after 14 days (Arzanlou *et al*., 2016).

Preparation of Soil Sample

River sand collected from river was obtained and brought to the laboratory for sterilization. The soil was autoclaved at 121°C and 15pound per square inch (PSI) for one hour, plastic pots (26 × 30cm) were filled with 7kg of sterilized soil. Sterilized plastic pots were perforated at the bottom to prevent water lodged. The soil in the pots were

watered once for three consecutive days in screen-house before planting (Halperin *et al.,* 2017).

Application Technique

Three different application methods were employed for the treatment viz: seed application of *Pseudomonas fluorescens,* foliar application of *P. fluorescens* and a combination of seed and foliar application method. At 47 days after sowing, groundnut plants were inoculated with a conidial suspension of *P. personatum* (5×10^4 spores mL^{-1}) (Meena*et al*., 2002).

Seed application

Groundnut seeds were soaked for two minutes with *Pseudomonas fluorescens* and seeds were sown (Meena *et al*., 2002).

Foliar application

Groundnut plants were sprayed with *Pseudomonas fluorescens* until runoff (Shifa 2018), on, 45, 60and 75 days after sowing using a low volume sprayer. The plants were also sprayed with mencozeb at, 45, 60and 75 days after sowing. Plants without *P. fluorescens* were used as a control (Meena *et al*., 2002).

Seed and foliar

Groundnut seeds were soaked for two minutes with *Pseudomonas fluorescens*. Seeds were sown (Meena *et al*., 2002) with some modification: use of gum arabic and then sprayed with *Pseudomonas fluorescens* until runoff (Shifa 2018), on 30, 45, 60, 75 and 90 days after sowing using a low volume sprayer. The plants were also sprayed with mencozeb at 30, 45, 60, 75 and 90 days after sowing.

Data Collection

The plant height and leaves number were counted an recorded and also number of branches/plant. The height of the plant was determined using standard meter scale or measuring tape by measuring aerial part of the plant from the soil surface to the terminal node of the developing leaf.

Experimental Design and data analysis

The study was arranged in a completely randomized design and each treatment was replicated four times. The data collected were subjected to analysis of variance (ANOVA). Means were compared through employing least significant difference (LSD) test at 5% probability level using MS Excel.

RESULTS AND DISCUSSION

The isolate of *Pseudomonas fluorescens* showed gram negative, rod shaped bacteria smooth and small colonies with cream and green pigment on Nutrient agar medium (Table 1). *Pseudomonas fluorescens* gave positive result for Catalase test, Gelatin liquefaction, Fluorescent pigment and Oxidase test but negative result for starch hydrolysis test. The result for the in vitro determination of antagonistic capability of *Pseudomonas fluorescence* against the growth of *Phaeoisariopsis personata* showed that *Pseudomonas fluorescence* had potential to inhibit mycelial growth of *Phaeoisariopsis personata* with percentage inhibition ranging from 64.70 to 70.58% (Figure 1).

The application method of *P. fluorescence* on plant height of some groundnut varieties infected with *Phaesariopsis personatum* is presented in Table 2. The result showed that at 45 DAS SAMNUT22 recorded statistically similar plant height to SAMNUT 24 and Jar gyada. At 60DAS SAMNUT 24 and SAMNUT 22 recorded the highest value in terms of plant height (35.30 and 32.65) respectively while Jar gyada recorded the least. At 75 DAS SAMNUT 22 recorded the highest plant height (43.20) followed by SAMNUT 24 and Jar gyada (38.20 and 36.95) respectively.

At 60 DAS standard showed the highest plant height (36.42) and the other treatments (control, foliar, seed, combined seed and foliar) showed statistically similar results. At 75 DAS standard recorded the highest (43.75), followed by combined seed and foliar recording 40.25 which is statistically similar to control and seed treatment of *P. fluorescence* while foliar recorded the least plant height although it was statistically similar to control and seed treatment. Results of the effect of different application method of *P. fluorescence* on number of leaves of some groundnut varieties infected with *Phaeoisariopsis personata* was presented in Table 3.The result showed that at 45 and 60 DAS the number of leaves are statistically similar for all the varieties SAMNUT 24, SAMNUT 22 and Jar gyada. At 75 DAS, combined seed and foliar treatment with P.

Fluorescence and seed treatment of P. fluorescence recorded the highest (67 and 65.67) respectively followed by standard and foliar (57.58 and 55.50) while the least (43.25) was recorded in control. At 75 DAS, combined seed and foliar treatment with *P. fluorescence*, standard and seed treatment of *P. fluorescence* recorded the highest (151.00, 140.67 and 137.50) respectively followed by foliar (129.67) while the least (116.33) was recorded in control.

Results of the effect of different application method of *P. fluorescence* on number of branches of some groundnut varieties infected with *Phaeoisariopsis personata* is presented in table 4. The statistical analysis showed that there is significant difference in varieties at 60 and 75 Days after sowing (DAS) but there is no significant difference at 45 DAS. At 45, 60 and 75 DAS there is significant differences across treatment. At 45 DAS seed treatment and combined seed and foliar recorded the highest number of branches (17.08 and 16.75) and the least was recorded by the control treatment (11.5) while at 60 DAS only control recorded the least 23.08, At 75 DAS standard and seed treatment recorded the highest (37.74 and 35.17) these were, followed by foliar and seed treatment (34.42 and 32.42) while the least value (29.25) was recorded in control.

In the present Study *Pseudomonas fluorescens* inhibited the growth of *Phaeoisariopsis personata* and the percentage inhibition over control was more than 60% and when the percentage inhibition radial growth (PIRG) was higher than 50% the antagonist is regarded as a promising biological control.

Comparing the method of application of *Pseudomonas fluorescens* to groundnut, combined seed and foliar application was found to be the best yielding better results in vegetative growth parameters tested and this is in agreement with the work of Prasad and Babu (2017) who compared the growth of *Azosppirillium brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogaea*). He found out that the color of the leaves and the overall plant growth were better in the *P. fluorescens* plant growth individually and in combination with *A. brasilense*.

Table 1 Morphological and Biochemical Characterization of Rhizobacterial Isolates

Characters	*Pseudomonas fluorescens*
Gram reaction	-ve
Cell shape	Rod
Pigmentation	Cream greenish
Colony morphology	Smooth margin
Catalase production	+ve
Oxidase	+ve
Starch hydrolysis	-ve
Gelatin liquefaction	+ve
Catalase	+ve
Fluorescent pigmentation	+ve

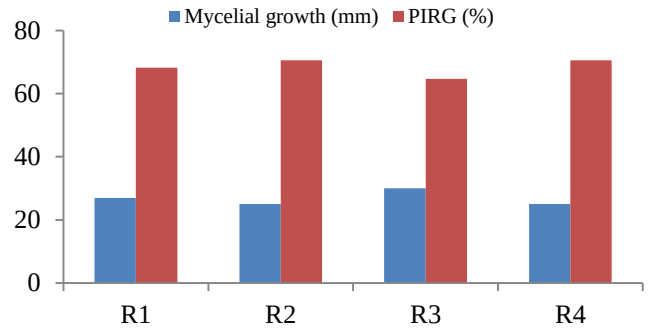

Figure 1: Effect of *Pseudomonas fluorescence* on the Mycelial Growth of *Phaeoisariopsis personata* under in Vitro Condition

Table 2: Effect of Different Application Methods of *P. fluorescence* on plant height of Groundnut Infected with *Phaeoisariopsis personata* at 47 Days After Sowing

Source of Variation	45 DAS	60 DAS	75 DAS
Varieties			
SAMNUT 24	27.55±3.0^{a}	32.65±2.8^{a}	38.20±3.8^{b}
SAMNUT 22	28.65±3.2^{a}	35.30±2.9^{a}	43.40±4.6^{a}
Jar gyada	25.80±3.2^{a}	31.75±2.2^{b}	36.95±2.5^{b}
LSD (P < 0.05)	4.59	2.81	3.89
Treatments			
Control	28.83±3.3	33.17±2.8^{b}	39.42±5.3^{bc}
Standard	28.17±3.6	36.42±2.0^{a}	43.75±4.9^{a}
Foliar	26.67±2.4	31.67±1.7^{b}	36.67±1.0^{c}
Seed	26.00±3.9	32.33±3.1^{b}	37.50±3.3^{bc}
Seed and foliar	27.00±2.9	32.58±3.2^{b}	40.25±4.0^{b}
LSD (P < 0.05)		2.26	3.13

Means with the same letter(s) in the same column are not statistically significant using LSD at 5% probability level. Key: DAS=Days after sowing, NS=not significant and *=significant

Table 3.: Effect of Different Application Methods of *P. fluorescence* on Number of Leaves of Some Varieties of Groundnut Infected with *Phaeisariopsis personata* at 47 Days After Sowing

Source of Variation	45 DAS	60 DAS	75 DAS
Varieties			
SAMNUT 24	58.60±13.4^{a}	102.90±17.5^{a}	136.8±17.0^{a}
SAMNUT 22	54.30±12.1^{a}	108.40±14.4^{a}	133.9±16.4 ^{a}
Jar gyada	60.50±13.3^{a}	108.20±8.9^{a}	134.4±13.9 ^{a}
LSD (P < 0.05)	10.91	10.94	NS
Treatments			
Control	43.25±6.5^{c}	91.83±19.0^{c}	116.33±8.8^{b}
Standard	57.58±16.8^{ab}	108.00±7.8^{a}	140.67±14.8^{a}
Foliar	55.50±7.7^{b}	105.83±7.7^{ba}	129.67±9.3^{ba}
Seed	65.67±8.6^{a}	115.33±11.1^{a}	137.50±8.3^{a}
Seed and foliar	67.00±6.9^{a}	111.50±9.9^{a}	151.00±11.2^{a}
LSD (P < 0.05)	8.79	8.81	14.23

Means with the same letter(s) in the same column are not statistically significant using LSD at 5% probability level. Key: DAS=Days after sowing, NS=not significant and *=significant

Table 4: Effect of Different Application Methods of *P. fluorescence* on Number of Branches of Some Varieties of Groundnut Infected with *Phaeisariopsis personata at 47 Days After Sowing*

Source of Variation	45DAS	60DAS	75DAS
Varieties			
SAMNUT 24	14.45±3.6^{a}	25.80±4.4^{a}	34.25±4.2^{a}
SAMNUT 22	14.35±3.4 ^{a}	27.35±3.6^{a}	33.55±4.1^{a}
Jar gyada	15.30±3.2 ^{a}	27.20±2.3^{a}	33.60±3.5^{a}
LSD (P < 0.05)	NS	2.91	3.59
Treatments			
Control	11.25±1.6^{c}	23.08±4.9^{b}	29.25±2.2^{c}
Standard	14.33±4.1^{b}	27.25±1.9^{a}	35.17±3.7^{a}
Foliar	14.08±1.9^{b}	26.67±1.9^{a}	32.42±2.3^{ba}
Seed	17.08±3.3^{a}	29.00±2.9^{a}	34.42±2.1^{ba}
Seed and foliar	16.75±1.9^{a}	27.92±2.5^{a}	37.75±2.8^{a}
LSD (P < 0.05)	2.60	2.34	2.89

Means with the same letter(s) in the same column are not statistically significant using LSD at 5% probability level. Key: DAS=Days after sowing, NS=not significant and *=significant

CONCLUSION

This study revealed that *Pseudomonas fluorescens* and *Phaeisariopsis personata* has been successfully isolated and *Pseudomonas fluorescens* showed positive result for all the biochemical test except starch hydrolysis which is negative.

This study revealed that *Pseudomonas fluorescens* effectively inhibit the radial mycelial growth of *Phaeoisariopsis personata* under in-vitro condition and this confirms the in-vitro biological activity of *P. fluorescens* towards *C. personatum* which is the causative agent of late leaf spot of groundnut.

Use of seed application followed by foliar spray of *Pseudomonas* enhance growth of *Phaeoisariopsis personata* of some groundnut varieties significantly. Increase in growth may be due to intrinsic ability of *P. fluorescens* to colonize the rhizosphere at a high density, to compete successfully with other microbes, and to produce secondary metabolite involve in plant growth stimulation as the result of rapid colonization of the rhizosphere by *P. fluorescens*. Therefore, Farmers should adopt the use of combined seed and foliar application of *Pseudomonas fluorescens* for effective control of late leaf spot of groundnut and for promoting the growth of groundnut.

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