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Research Article

Evaluation of rhizobia inoculants and fungicides in the management of fusarium wilt disease of cowpea (*Vigna unguiculata* (L.) walp)

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Abstract: The antifungal potential of Rhizobia inoculant (Ri) and its compatibility with fungicides in controlling Fusarium oxysporum f. sp. tracheiphilum (FO) in cowpea was investigated. Fifty cowpea nodules were collected from fields in Kano and Ibadan, isolation, authentication and identification of Rhizobia were carried out. Antagonistic potential of best two strains (R2 and R3) were tested in-vitro on FO with USDA 2677 (R1) as reference. Fungicides and Rhizobium compatibilities were carried out on seeds dressed with the four fungicides. Seeds were thereafter coated with R1, R2 and R3 and rhizobium strains. Seven local strains (Ibadan1, 2 and 16, Kano18, 19, 42 and 49) were identified as Bradyrhizobia spp. In terms of number of nodules, Ibadan 16 (9) and Kano 19 (7) performed better, compared with Reference Rhizobium (11). Ibadan16, Kano19 and R3 significantly inhibited mycelia growth of FO. The TMD (6.33E +08), IMC (6.25E+08) enhanced growth than MC (4.30E+08) while CM (0.00) inhibited growth of all Rhizobium strains, with control having highest (3.89E+10) CFU/mL. Thiamethoxam + metalaxyl-M + Difenoconazole and Imidacloprid + Metalaxyl + Cabendazim were compatible with Ibadan 16 and Kano 19. Indigenous Rhizobium competed well with exotic strains (USDA 2677) and are recommended for use in cowpea inoculant production.

Keywords: Fusarium oxysporum f. sp tracheiphilum, Rhizobium strains, fungicides, Antifungal potential

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an important grain legume which is commonly cultivated in the sub-tropical and tropical areas of the world. It is a multipurpose crop grown for both humans and livestock's and it plays crucial roles to many relatively poor African and people in other areas in the developing countries (FAO, 2022). Symbioses occur between cowpea and root nodule-forming bacteria (rhizobia). Rhizobia are group of soil inhabiting micro-organisms, which possess the ability of fixing nitrogen on leguminous crops, thereby reducing the quantity of synthetic nitrogen fertilizer required to little or nothing (Herridge et al., 2008). They enhance plant development directly by fixing nitrogen, increasing nutrient availability, producing phytohormones, and solubilizing minerals, or indirectly by acting as natural antagonists that restrict the growth of pathogens (Figueiredo et al., 2010). In any of these functions, rhizobia are a very essential component of an ecosystem.

Constraints to obtaining high yield of cowpea in the sub-Saharan Africa are biotic and abiotic factors. Biotic factors include insect pests and pathogenic organisms while abiotic factors include low soil fertility, heat, drought, excessive moisture, inadequate management and expensive technology unaffordable for peasant farmers (Killani et al., 2011). Pathogenic organisms account for a lot of crop production losses in tropical agro-ecosystems. Soil borne pathogens associated with cowpea include Macrophomina phaseolina, Fusarium spp, Colletotrichum spp, Pythium aphanidermatum, Xanthomonas spp, Sclerotium rolfsii and Rhizoctonia solani. These pathogens have very high tendencies to

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cause damping off in seedlings' pre-and post-emergence of cowpea, resulting in diseases that cause drastic yield reduction to cowpea crop (Peluola & Fadina, 2014; Boukar et al., 2019). In modern day agriculture, integrated pest management (IPM) which includes planting of improved and resistant varieties, application of chemical pesticides, good sanitation procedures, biological control methods and good agronomic practice in general are measures employed in ensuring cowpea production is optimised (Stenberg 2017). The fundamental principle of managing an ecosystem emphasizes the importance of every component working together to produce a sustainable balanced system (O'Connor et al., 2019). The concept of ecosystem should be understood before any external input aiming at improving crop productivity is introduced because, the complex interactions that exist among organisms in the ecosystem require the need to guide both the level and form of interference introduced to it by man (Omoloye, 2008).

Seed dressing with fungicides has been extensively used to prevent wilting of cowpea plants, but as a result of their indiscriminate use, agrochemicals generally are becoming less preferable, due to environmental concerns (Adegbite & Amusa, 2008). Also, certain chemicals which are used in controlling pests have broader scope of biological functions which act more than the indicated mode of action as prescribed by the producer; this could result in indirect non-target effect on other organisms in the ecosystem (Jacqueline et al., 2011).

Different organisms in an ecosystem may possess identical or similar cellular composition, therefore, non-target organisms may be adversely affected by fungicides that target non-specific binding sites (Noel et al., 2022). Microorganisms are also intertwined either in function or in their nutritional requirements, thus, any change in the components of a microbial community can have an impact on the overall functionality of that community. This is relevant to plants which have link with microorganisms that impact and are impacted by the environment (Yang et al., 2011).

Cowpea productivity can be enhanced through the application of rhizobium inoculants specifically formulated for cowpea. Some Rhizobium strains also possess antagonistic potential against certain pathogenic microorganisms. The common practice among farmers is to apply fungicides as seed dressing on cowpea seed to prevent wilting during pre and post emergence of cowpea seedlings (Gomes et al., 2017). There are uncertainties about fungicides combination with rhizobia inoculants, as the combination with fungicide could pose toxic effect on rhizobia cells and this may affect their effectiveness (Santos et al., 2022). However, there is limited information on the interactivity between rhizobia and fungicides in cowpea production. Therefore, it is important to investigate the impact of fungicides on beneficial activities of rhizobia when cowpea seeds are coated with both fungicides and rhizobia inoculant.

The study was therefore undertaken to investigate how the application of fungicides to manage the incidence of Fusarium wilt can affect the performance of rhizobia inoculant, when cowpea seeds are coated with both legume inoculant and fungicides.

2. Materials and methods

The experiment was carried out in the laboratory and screen house of Soil Microbiology Unit of the International Institute of Tropical Agriculture (IITA), located at 7° 22' 36.2496'' N and 3° 56' 23.2296'' E in Local Government Area of Akinyele, Ibadan, Oyo State. Characterized Fusarium oxysporum f. sp. trachephilum culture was collected from the Germplasm Health Unit IITA. The reference rhizobium strain for cowpea (USDA 2677) was obtained from the Soil Microbiology Unit, IITA Ibadan. Cowpea variety TVU 3192 was obtained from the IITA Cowpea Breeding Unit. The fungicides tested include: Seed plus (Imidacloprid 10% + Metalaxyl 10%+Cabendazim 10% WS), Apron star (20% w/w thiamethoxam+ 20% w/w metalaxyl-M +2% difenoconazole) and Ridomil (6% methalaxyl -M and 60% copper in form of cuprous.

2.1. Isolation of rhizobia from root nodule

Root nodules were collected from the roots of cowpea in farmer's field from Kano and seed production fields at Ibadan with no previous history of inoculation. These were kept in vials with screw caps, transferred to the laboratory and preserved in the refrigerator. The nodules were surface sterilized with 95% ethanol for 10 sec, then with 3% NaOCl solution for 3 min and sterile distilled water was used to rinse the nodules five different times. The nodules were pressed with blunt-edged glass rod and the suspensions obtained from crushed nodules were streaked on Yeast Mannitol Agar (YMA) plate. The cultures were kept in the incubator for 5 days at 28 °C and plates were checked for distinct colonies that looked milky, dome or flat shape. Further subculturing was done by streaking on YMA to obtain pure cultures (Woomer et al., 2010; Somasegaran & Hoben, 2011). Presumptive test was carried out on the isolates that were typical of rhizobia, based on their shapes, size color and texture of colonies. The isolates were further subjected to molecular identification and

characterization by obtaining whole genomic DNA from the rhizobia samples using the ZYMO kit (Zymo research group California, USA).

A total of six isolates were selected for the direct sequencing of PCR fragments obtained, using the primer 16S rRNA PCR 27F: AG-3'; 1492R: 5'-GGTTACCTTGTTACGACTT-3'. Results from the OTU clustering were compared to the Greengenes data base using an RDP classifier within the QIIME pipeline and used for further analysis.

The identified rhizobia were authenticated in the screen house by planting pre-germinated cowpea seedlings with regular growth in 5 kg pots already filled with sterilized ocean sand at the rate of one seedling per pot. The plants in the labelled pots were inoculated with 1 mL of the broth cultures for each of the isolates and the reference strains at seven days after planting (DAP). The un-inoculated plants present as control and the plants were kept moistened with sterile distilled water as necessary. The plants were examined from 15 Days after planting (DAP) for contrast in vigour and colour between the inoculated treatments and un-inoculated treatment. At 4 weeks after planting (WAP), the plants were uprooted, and the roots were checked for the presence of nodules. The treatments with nodules were scored as positive (+) and ones without nodules were scored as negative (-). For the purpose of this study, only 2 isolates were selected based on their level of infectivity in terms of number of nodules and plant vigour.

Peat based semi-solid inoculant was prepared with selected strains and were left to cure for 2 weeks at 28 °C. Spore suspension of Fusarium oxysporum was prepared from a week-old culture. Suspension was passed through sterile muslin material and the concentration of the final suspension was diluted from 1.0 x 108 spores/mL to 1.0 x 106 spores/mL. Haemocytometer was used for the microscopic enumeration (Aberkane et al., 2002).

2.2. Preparation of Fusarium oxysporum f. sp. trachephilium inoculum

A week-old culture of F. oxysporum f. sp. trachephilum was harvested by covering the fungal colonies with 5 mL sterile distilled water and the top part was removed with a sterile scalpel into 45 mL sterile distilled water in 100 mL conical flask. Suspension was passed through sterile muslin material and the concentration of the final suspension was diluted from 1.0 x 108 spores/mL to 1.0 x 106 spores/mL. Haemocytometer was used for the microscopic enumeration (Aberkane et al., 2002).

2.3. Pathogenicity test of Fusarium oxysporum f. sp. tracheiphilum

This test was conducted to confirm the pathogenicity of F. oxysporum f. sp. tracheiphilum, the causal organism of fusarium wilt of cowpea. Soil was distributed into 1 kg transparent autoclavable polyethylene bags and sterilised in the autoclave for 1 hour at 121°C under 15 kg/cm pressure and was allowed to cool overnight, after which it was filled into 2 kg pots in the screenhouse. Cowpea seeds were surface sterilized with 3% hypochlorite solution for 1 min, then the seeds were rinsed with sterile distilled water five times. Four seeds were sown and were thinned to two seeds per pot. The spore suspension of Fusarium oxysporum f. sp. trachephilum was prepared and the suspension was adjusted to 1 x 106. At 7 DAP, the plants were inoculated by trimming the lateral root of the plants with sterile pair of scissors and dipping the root part of each plant into 100 mL spore suspension for 60 s. Inoculated plants were re-planted into the same pots from which it was uprooted initially (Kunta et al., 2015). The control plants were dipped into ordinary sterile distilled water and plants were maintained in the screenhouse. After 4 WAP, inoculated plants showed typical wilt disease.

2.4. In-vitro screening of Rhizobia strains for antifungal potential

Two local strains of Rhizobia, Ibadan16 and Kano19 with the reference strain USDA2677 were tested for antagonistic ability against Fusarium oxysporum on PDA medium by the dual-culture plate method. The experimental design was a complete randomized design (CRD), and the treatments were replicated three times. For each of the three strains, 5 mm disc of pure culture of the F. oxysporum f. sp. trachephilum was set at the middle of a 90 mm Petri dishes and rhizobia strain was inoculated near the edges of the PDA plate at four equidistant points. Fusarium oxysporum f. sp. trachephilum only was set at the middle of another Petri dish and this served as control in comparing the level of inhibition in the treatment plates. Plates were incubated at 28°C for 5 days. The diametric growth of the antagonist and zone of inhibition were measured, results were recorded as the mean inhibition growth (Odebode et al., 2004).

Percentage (%) growth inhibition was calculated as:

$((Dc - Dt) / Dc) \times 100$

Where Dc=Diameter of the pathogen in the control plates

Dt = Diameter of the pathogen in the treatment plate

2.5. In-vitro screening of the compatibility of selected Rhizobia with selected fungicides

The four selected fungicides were tested with the reference rhizobium strain and the isolated indigenous strains. The experiment was a two-factorial complete randomized design (CRD) and each treatment was in three replicates. Cowpea seeds were weighed with a weighing balance Mettler Toledo (model MS 16025) into clear polyethylene bags for different concentration of fungicides (within the range of manufacturer's recommendation for each of the selected fungicides; Seed plus 2.5 g/ kg, Ridomil 3.3 g/ L, Apron star and 2.5 g/ kg and Teem 10 g/ kg of seeds. The seeds were first coated with fungicides and immediately with rhizobia inoculant at the rate of 10 g/kg of seeds. The three rhizobia inoculant were used to coat the seeds separately and that served as the controls for each treatment combinations. A quantity of 5 mL sterile distilled water was prepared in vials and sterile forceps was used to pick five previously coated seeds into the sterile distilled water in the vials. The mixture was mixed with vortex mixer for 1 min after which 1 mL of the aliquot was picked into a 9 mL sterile distilled water in a vial. Tenfold serial dilution was prepared up 10-7 and aliquots of 10-4 – 10-7 were plated on Congo red agar immediately after dilution (starting point) using drop plate method with three replications and the stocks in vials were kept in the refrigerator at 4 °C for 48 hr. after which another tenfold serial dilution was prepared, and the aliquots plated on Congo red agar. Plates were kept in the incubator at 28 °C for 3 days, and then rhizobia colonies were recorded. Colony forming unit /ml (CFU/mL) at different concentration of fungicides was calculated as: No of colonies ^{x} vol. of aliquot used ^{x} Dilution Level (Somasegaran and Hoben 1994).

2.6. In-vivo screening of the compatibility of selected Rhizobia with the selected fungicides.

The experiment was conducted at the screenhouse in IITA Ibadan. Soil collected from Kano State was sterilized and filled into 5 kg pots in the screenhouse. Half strength N-free nutrient solution was prepared and sterilized, 50 mL was added to the soil before planting. The experimental design was 3 by 2 factorial in CRD with four replications. The cowpea variety TVU 3192 was selected for use because of its susceptibility nature to fusarium wilt. The seeds were coated first with fungicides at the rate of 2.5 g per kg of seeds and afterward inoculated with peat-based inoculant prepared with the selected strains at the rate of 10 g per kg of seeds; the adhesive of choice is gum Arabic. Seeds were planted into labeled pots at the rate of four seeds per pot and thinning was done at 5 DAP to two plants per pot. At 7 DAP, the plants in pots numbers T 1- T 6 and T 11 were inoculated by uprooting and trimming the lateral root of the plant with sterile scissors and dipping the root part of each plant into 100 mL spore suspension of Fusarium oxysporum f.sp. tracheiphilum adjusted to $1^{x}106$ for 60 s. Inoculated plants were re-planted into the same pots from which they were uprooted initially (Kunta et al., 2015). Pots labeled T 7 – T 9 were planted with seeds inoculated with inoculant prepared with rhizobia strain USDA2677, Ibadan16 and Kano19 respectively without fungicide application. The pot with cowpea only (T10) presented as control for the first experiment while T11 was only included in the second experiment to further compare the treatments with spore suspension of F. oxysporum f.sp. tracheiphilum + rhizobia strains and the treatment with spore suspension of F. oxysporum f.sp. tracheiphilum only. Plants were kept moistened with sterile water when necessary and the experiments were terminated at eight WAP.

The factors are as follows: Three Rhizobia strains; USDA2677, Ibadan16 and Kano19: two fungicides; Apron star and seed plus Treatments Keys and combinations: F1 - Apron Star, F2 - Seed Plus, St1 - USDA 2677, St2-rhizobium strain 16, St3 - rhizobium strain 19 and Cowpea variety - TVU 3192. The treatments were combined as: T1: fungicide 1 + strain 1, T2: fungicide 1+ strain 2, T3: fungicide 1+strain 3, T4: fungicide 2 + strain 1, T5: fungicide 2 + strain 2, T6: fungicide 2 + strain 3, T7: strain 1 only, T8: strain 2 only, T9: strain 3 only, T10: control 1 (plant only) and T11: control 2 (Fusarium oxysporum f.sp. tracheiphilum + plant).

2.7. Data analysis

Data was analysed using Statistical Analysis System (SAS) version 9.1 and dependent variables were subjected to Analysis of Variance (ANOVA).6. The Least Square Means (LSD) test at P = 0.05 was used to compare treatment means.

3. Results

3.1. Isolation, presumptive test, and authentication of Rhizobia strains

Twenty isolates were obtained from the isolation of rhizobia from nodules of cowpea, nine isolates were Gram-negative bacteria. The authentication test,

confirmed that only five isolates formed root nodules and were rhizobia. Out of the five confirmed rhizobia isolates, Kano19 and Ibadan16 showed high level of infectivity. Plants treated with Isolate Ibadan16 had the highest number of nodules, followed by isolate Kano19, they also showed good vigor and were greener. They compared well with the plants inoculated with reference strain (USDA2677), and the inner part of their nodules showed pink colorations. Plants treated with other isolates did not perform very well in terms of nodule number and vigor; no nodule was present on the root of the control plants.

3.2. Quality of the extracted De-oxyribonucleic acid (DNA) and its amplification

The DNA from the bacteria was found to be present after extraction, hence the thick band upon loading of DNA on gel. The DNA was successfully amplified using the 27F an1492R primers having a band of about 750 kbp. The amplicons showed that, they could be used for sequencing as they had sufficient concentrations with the right or expected band sizes. Also, it was confirmed that they were bacteria species, as 16S is used to target the common housekeeping genetic marker which is found to be present in almost all bacteria.

3.3. Molecular identification of Rhizobial isolates

Blasted sequence result showed that, rhizobia Kano49, Ibadan1 and Kano19 had higher percentage similarity with Bradyrhizobium diazoefficiens. USDA2677 was identified as Bradyrhizobium species. Clone Vs J5W1U24, and Bradyrhizobium species. Clone VPK55w1Uo1 which they were 100% identical with, while Rhizobium Ibadan16 was identified as Bradyrhizobium species. H4R5pA and Bradyrhizobium species. H2R4pA as it was 100% identical with them. Rhizobia strain 49 and 1 were much closer to each other than to Kano19 (Figure 1).

3.4. In-vitro screening of selected Rhizobia strains for antagonistic potential against Fusarium oxysporum f. sp. Tracheiphilum

Isolate 19 had the highest mean (50.03) for percentage zone of inhibition, followed by the reference strain (USDA2677) (45.02) and the least was isolate 16 (44.80). The control plate with Fusarium oxysporum f. sp. tracheiphilum (0.00) alone was significantly different from the other three treatments that had different rhizobia strains paired with them at $P = 0.05$. The rhizobia strains tested inhibited the growth of Fusarium oxysporum f. sp. tracheiphilum at different extents. However, there was no significant difference among the three pairs (Table 1).

3.5. Effect of different concentrations of selected fungicides on the growth of selected Rhizobium strains (CFU/mL) at different inoculation time

The results obtained showed that the treatment without any fungicide (control) had the highest mean value and was significantly different from other treatments, both at inoculation and 48 hr. after inoculation. This was followed by a 2.5 g/kg concentration, which was also significantly different from the 3.3 g/kg and 10 g/kg, which were not significantly different from each other (Figures 2 and 3).

3.6. Effect of different fungicides on the cfu/mL of selected Rhizobium strains

When USDA2677 was combined with the four selected fungicides, at the point of inoculation, the combination of USDA2677 with Apron star (8.3 x 10^7) and Seed plus (8.2 x 10^7) had the highest mean values and were not significantly different from each other. This was followed by the treatment USDA2677 with Teem (4.3 x 10^7) which was also significantly different from the combination of the strain with Ridomil (0.0). The combination of USDA2677 with Ridomil showed no growth at all levels of the concentration tested. The same trend was also observed for all treatments at 48 hrs when combined with USDA2677. In the case of isolate 16, for the four selected fungicides at the point of inoculation, the highest mean value was recorded with Seed plus (4.8 x10^7). However, it was not significantly different from the response obtained with Apron star and Teem (4.3 x10^7) and (4.2 x10^7) respectively, but these were significantly different from Ridomil. At 48 h, combination with seed plus (8.2 x10^7) was significantly different from the three other fungicide treatments, this was followed by Apron star (6.1 x10^7) which was significantly different from Teem (3.8 x10^7) and Ridomil. Teem, in turn, was significantly different from Ridomil (0.0) for which no growth was observed. It was also observed for isolate 19 that, for the four selected fungicides, at the point of inoculation, the treatment combination with Teem (5.9 x10^7) had the highest mean value and it was significantly different from Seed plus (4.5 x 10^7), which came next in ranking. This was also significantly different from Apron star (2.9 x 10^7), while Ridomil (0.0) did not support the growth of the strain. At 48 hours, however, there was no significant difference among all treatments except for the treatment

combination with Ridomil, which showed no growth (Figure 2 and 3).

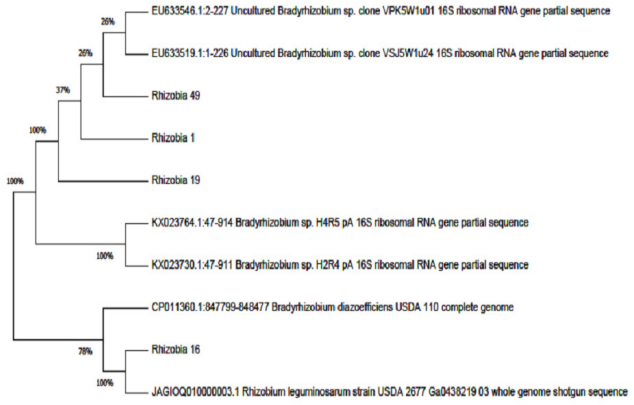

Figure 1: Phylogenetic tree for the selected Rhizobium strains

Table 1: Antagonistic effect of selected Rhizobia strain on F. oxysporum f.sp. tracheiphilum based on percentage zone of inhibition

Strains	Means of percentage zone of inhibition
F. oxy	0.00±0[b]
F.oxy + USDA 2677	45.02±10.2[a]
F.oxy +19	50.03±1.9[a]
F.oxy + 16	44.80±2.2[a]

Means with the same letter are not significantly different at p ≥ 0.05

Effect of different concentrations of selected fungicides on the Colony forming units/mL of Rhizobium strains at inoculation and 48 hr. after inoculation (CFU/mL)

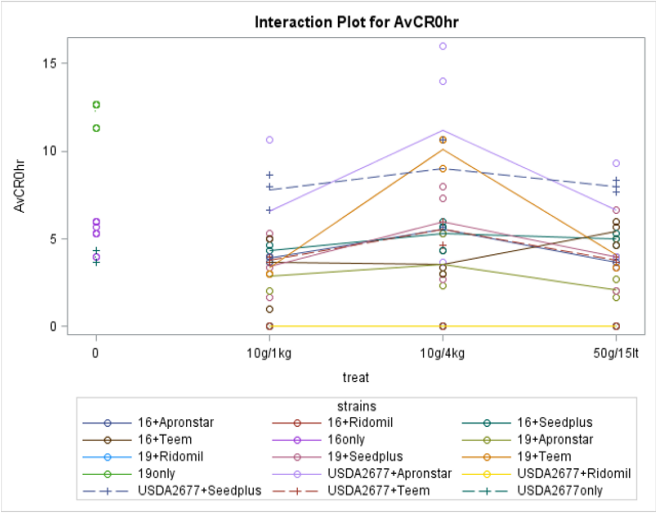

Figure 2: Interaction of selected fungicides and Rhizobium strains at different concentrations of fungicides at the point of inoculation

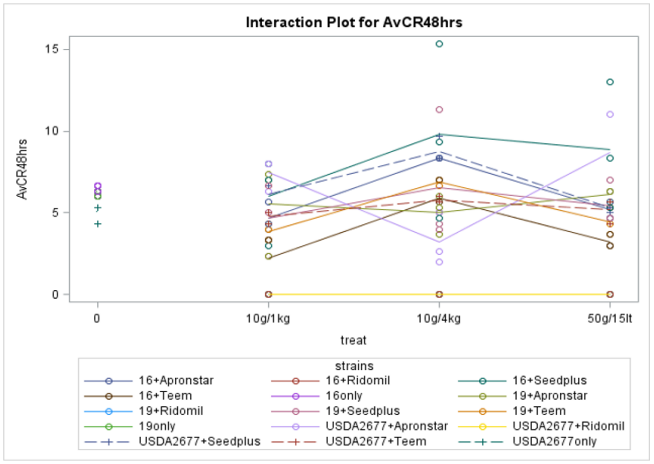

Figure 3: Interaction of selected fungicides and Rhizobium strains at different concentrations 48 hours after inoculation

4. Discussion

The compatibility of fungicides and rhizobia inoculant has been an important question by farmers who seek to improve their livelihood and meet the food needs of the increasing world population. Smallholder farmers rarely have access to mineral nitrogen fertilizer due to issues bordering on its availability and affordability. Hence, they are concerned about protecting the plants from phytopathogens, at the same time seeking to maximize the benefits of the symbiosis which exists between legumes and the beneficial bacteria (rhizobia) in the soil.

The two isolated local strains Ibadan16 and Kano19 were identified as Bradyrhizobia species and they had higher similarities with Bradyrhizobia diazoefficiens. This is in agreement with Guimarães et al. 2012; Jaramillo et al. 2013; Ndungu et al. 2018 who found that most rhizobia strains associated with cowpea are Bradyrhizobia species. According to Santos et al. (2022), cowpea is a promiscuous grain legume that is normally nodulated by Bradyrhizobium species, which exhibits slow-growing properties.

The three tested strains USDA 2677, Ibadan16 and Kano19 against Fusarium oxysporum f.sp. tracheiphilum suppressed the growth of Fusarium oxysporum f.sp. tracheiphilum on the PDA medium at different level. This corroborated the submission of Kucuk and Cevher (2015) that some rhizobium strains suppressed mycelia growth of fungi due to the synthesis and production of siderophore. Akhtar (2014) in his study also opined that, some bacteria were proven to support plant development by activating the blossoming of secondary roots, serving the purpose of defense against plant pathogenic microorganisms aided by plant growth regulators such

as auxin, gibberellin, siderophore, HCN and antibiotic secretion.

As for the colony forming units obtained (CFU/mL) at different inoculation period, this had no regular pattern of effect among the strains when the seeds were initially coated with both fungicides and the strains and after 48 h. of inoculation. However, the control treatment had the highest rhizobia count both at the time of inoculation and 48 hr. after inoculation. This is in accordance with the submission of Mishra et al. (2013) who stated that, the population of the beneficial microorganism tested significantly reduced when co-treated with tested fungicides.

The performance of rhizobium strains alone was better, compared with when paired with the following fungicides; Apron Star, Seed Plus, Ridomil and Teem at the manufacturers' recommended doses. The dose of 2.5 g of fungicide per kg of seed which was the lowest concentration supported the growth of all the rhizobia strain more than other concentrations. This is in support of the study carried out by Ahamed et al. (2007) which opined that, fungicides in the lowest recommended doses had mild effect on the cells of rhizobia and generally, rhizobia or bradyrhizobia, either exotic or indigenous act differently in their response to the tested fungicides. It was also observed that, among all the four fungicides tested, there were growth of the rhizobia strain at varying cfu/ml, except that none of the rhizobia strains was compatible with Ridonmil at all concentrations as no growth was observed on the Congo red plates. Ramos and Ribero (1993) earlier found that, application of Ridomil as one of the fungicides he tested in his study caused drastic mortality on two strains of rhizobium. It was further confirmed that, ridomil may not be compatible with rhizobia strain independent of the time of seed dressing as Martyniuk et al. (2002) found that, when mancozeb and ridomil were both tested on two rhizobia strains at inoculation and 24 h after inoculation, one of the strains responded to mancozeb whereas, ridomil had negative effect on both rhizobia strains.

5. Conclusion

This study showed that the two indigenous rhizobium strains (Ibadan16 and Kano19) compared favorably with the reference strain USDA2677 in terms of colony-forming units (CFU). In addition, all three strains demonstrated antagonistic potential against Fusarium oxysporum f. sp. tracheiphilum, the causal agent of Fusarium wilt in cowpea.

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