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EFFECTS OF *RHIZOBUIM LEGUMINOSARUM* INOCULATION ON THE GROWTH AND YIELD OF *MUCUNA FLAGELLIPIES*

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ABSTRACT

The effects of *Rhizobium leguminosarum* inoculation on the growth and yield of *Mucuna flagellipies* (Vogel ex Hook) was conducted for two years (2008 and 2009) in the Greenhouse, Department of Agronomy, Obubra, Cross River University of Technology, Cross River State. Treatments were three *Rhizobium* strains (CB188, CB756, IAC636 inoculants and no *Rhizobium* (control), inoculated on *Mucuna flagellipies* seedlings planted in the polyethelene bags (50x30cm) arranged in a Completely Randomized Design with eight replications. The results showed that all cases of inoculation with *Rhizobium* strains significantly ($p<0.05$) increased higher growth and yield compared with the non-inoculated ones. *Rhizobium* inoculated plants produced more number of leaves per plant (28.4 and 29.3), branches (5.3 and 6.2), longest vine length(225.5 and 238.3cm) than the non inoculated at 50% flowering in 2008 and 2009 seasons respectively. The highest leaf area index value (4.03 and 4.06), biomass dry weight of plant fractions; nodule (0.78 and 0,79g), vine(11.32 and11.44g), root (6.51 and 6.51g),and leaf (14.5 and 15 2g) were obtained in plants inoculated with IAC 636 *Rhizobium* strains. Inoculation also promoted earlier flowering ,pod formation in *Mucuna flagellipies* as compare with the non inoculated ones .Similarly, the same strain (IAC 636) gave the best seed yield of 120.0g and 126.1g per plant in 2008 and 2009 seasons, respectively. The findings suggested that inoculation of *Mucuna flagellipies* with Rhizobium is beneficial and produced high seed yield and could be use as biofertilizer an alternative to nitrogen fertilizer.

KEY WORDS: *Mucuna flagellipies, Rhizobium*, Inoculation, Growth, and Yield.

INTRODUCTION

Mucuna flagellipies (Vogel ex Hook) is of the Fabaceae family, sub-family Papilionoideae (Polhill and Raven 1981). It is a legume, climbing perennial herb with compound trifoliate leaves that is indigenous to Nigeria (Anonymous 1979). *Mucuna flagellipies* is one of the less known legumes with high protein value. The seed is rich in protein, edible oils, fats and mineral (Odedele ,1983). It is widely consumed among the Igbo-speaking people in soup where it performs the basic function of soup thickener (Oyenuga ,1986; Agba, *et al*., 2005).

Both the seed and leaf of *Mucuna flagellipies* have high economic value in pharmaceutical industry and other domestic uses.. In pharmaceutical industry, the gum produced from the seed could be used as a binder in the formulation of epherdrine hydrochloric tablet (Chukwu ,1986; Eyiuche 1988 and Okon 1989). The leaves are used to formulate local hair dye (Okoro, 1989). *Mucuna flagellipies* is variously call as" Ukpo", 'Ibaa', or Okobo by the Igbos of eastern Nigeria. The Hausas call it "Karangiwa', the Yorubas call it "agbarin" while the Efiks call it "Ibaba" (Oyenuga, 1986).

Despite the economic importance of this crop, it is grown only on a sub-subsistence level mostly as a compound crop by some Igbos of South East Nigeria. There is paucity of information on the growth of the crop in a commercial scale .This formed the baseline of this study.

In many agro-ecosystems, nitrogen input has become one of the vital elements to achieving higher yield (Hardy, 1993). Nitrogen is a unique element and the most common limiting element for plant growth, biomass production, agricultural productivity and one of the most expensive to purchase as fertilizer and non of it forms exist as mineral in the soil (Elkany, 1992).

Traditionally soil nitrogen deficiency has been addressed by applying mineral fertilizer (Henzell, 1998). Although, it is recognized that nitrogen fertilizer are largely responsible for increase in crop yield, there remains a wide gap in fertilizer use between the developing and developed countries (Kaweski, 1994). In spite of much investment in research and advances in fertilizer management, most crops utilize less than 40% of the applied nitrogen (Henzell, 1998). This is mainly because of nitrogen losses, that occur through ammonia

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(NH_3) volatilization, leaching, plant removal and denitrification on the surface of the soil and in the rhizophere (root zones), (Yanan, 2003; Brockwell *et a,l* 2004).

Studies by Danso and Owiredo, (1998), Dick (1999) and Yanan (2003) indicated that introduction of beneficial symbiotic microorganisms into the plant Rhizosphere (inoculation) has been shown to improve plant growth and yield. Inoculation of plants with effective *Rhizobium* strains has produced better nodulation and nitrogen fixation in poor soils (Kaweski 1994). Dick(1992) report significant increased in soybeans vegetative growth measured as leaf area index (LAI) as affected by inoculation with Bray *Rhizobium japonicum* Similarly Henzell (1998) working on pot experiments obtained significantly higher crop growth rate and taller soybean plants in Rhizobium inoculated plants than the non inoculated ones. Safir et al (2003) recorded better pod and seed yield in Rhizobium inoculated peanut plants than others. They attributed the increased in yield to earlier flowering, pod set and pod filling by Rhizobium inoculation that enhance higher nutrient availability and uptake.

There is scanty literature information on the *Rhizobium* inoculation effects on the production of *Mucuna flagellipies*, a legume with high nutritional value and nitrogen fixing capacity that is under utilized. Therefore this study aimed at investigating the effects of *Rhizobuim* inoculation on the growth and yield of *Mucuna flagellipies* in the greenhouse.

MATERIALS AND METHODS

Experimental Location.

Studies were carried out at the greenhouse of the Department of Agronomy, Cross River University of Technology, Obubra campus in two years 2008 and 2009. Obubra is located at latitude 05^0 59'N and longitude 08 16" E. The annual rainfall, relative humidity and temperature ranged between 2200-2500mm, 76.8-87.6% and 30.3-33 .0^0 C, respectively (CRADP 1992).

Experimental Design:

The experiment was a completely randomized design (CRD), treatments consist of three *Rhizobuim* strains inoculants collected from the Fertility Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan Oyo State and uninoculated control with eight replications. The three *Rhizobium*(*Rhizobium leguminosarum)* strains were CB 188, CB 756, IAC 636 . the experimental sizes was 24.5m x4m with an area of $98M^2$. This was divided into eight replications that contain eight plots. Each plot size was 2m x1.5m with an area of $3M^2$ the sampling areas size was 1.5m x 1.4m ($2.1m^2$).

Preparation of *Mucuna flagellipies* seedlings for inoculation.

Mature healthy seeds of *Mucuna flagellipies* were collected from Ikom central market, sorted, washed with 95% ethanol and surface sterilized with 3.5% sodium hypochlorite .Thereafter the seeds were rinsed three times with distilled water and planted out in sterilized germination troughs underlined with well watered cotton wool. The planted seeds were watered regularly and monitored for germination.

PREPARATION OF PLANTING MEDIUM

Top soil and river sand were sterilized in an autoclave at 121 ^{0}C for 30 minutes. then mixed with well cured poultry manure in a ratio of 3:2:1 of top soil:
Poultry manure: river sand. Five (5kg) of the sterilized soil medium was transferred into perforated plastic ployethelene bags of 50cm x 30cm ($150cm^3$). The polyethelene bags were watered for three days before transplanting of *Mucuna flagellipies* and *Rhizobium* strain inoculation.

PREPARATION OF RHIZOBIUM INOCULANTS FOR INOCULATION OF *MUCUNA FAGELLIPIES* SEEDLINGS.

Three *Rhizobium* (*Rhizobium leguminosarum*) strains (CB188, CB756, IAC 636, and zero or no *Rhizobuim* strain (control) inoculants collected from the Fertility laboratory, International Institute of Tropical Agriculture (IITA) were streaked from the stock agar culture into separate freshly prepared sterile Potato Dextrose Agar (PDA) medium and incubated at temperature of 36-37^0 C for seven days . Thereafter, the *Rhizobium* inoculants were transferred from the incubated agar culture to separate freshly prepared sterile yeast manitol broth mounted on a wrist-action shaker and allowed to shake until visibility through the broth was zero.

Seven days after *Mucuna flagellipies* seedlings were transplanted into the polyethelene bags, appropriate *Rhizobium* strains in the yeast manitol broth were inoculated at the base of each plant using sterile syringes at the rate of 2ml per seedling and then washed down the soil with 5ml of distilled water. The inoculants contained sixteen (16) colonies of *Rhizobium leguminosarum* bacterial cells per milliliter (ml) of the yeast Manitol broth. Seedlings were inoculated because *Mucuna flagellipies* seeds have very hard seed coat and dormancy problem that hinder germination and uniform field establishment within a short period of time . The plants were not watered for 3-7 days to avoid water washing away the Rhizobium inoculants from the seedlings. And when watering resumes, it was done cautiously and at the periphery of the polyethelene bags to avoid splashing out of *Rhizobium* inoculants. Thereafter, watering was done regularly at two days intervals. The seedlings were guided on the trellises' for support and monitored for data collection.

DATA COLLECTION.

Date were collected on the following variables: number of leaves and branches per plant, height (length of longest vine cm), Days to first and 50% flowering, pod and seed yield per plant.

Growth Analysis

Growth was evaluated through destructive sampling of plants from the sampling area and records taken on growth parameters (leaf area, leaf area index and dry weight of plant fractions).

METHODS OF DATA COLLECTION AND DETERMINATION OF GROWTH PARAMETERS

Number of leaf Blade and Branches per plant.
At 14 weeks After planting (WAP) when *Mucuna flagellipies* plant canopy (leaves and shoots) were fully spread and well developed and 50% flowering, the number of leaf blade per plant from all plant in the sampling area ($2.1m^2$) in each treatment were counted "insitu" and the means calculated to the nearest whole number and recorded as the number of leaf blade per plant. The same procedure above was used to determine the number of branches per plant.

Plant height (length of longest vine in cm).
Mucuna flagellipies plant height was determine as length of the longest vine, using all the plant in the sampling area ($2.1m^2$) in each treatment. Each plant was measured from the ground stretching the measuring tape to the terminal end of the plant. The means were calculated to the nearest centimeter (cm) and recorded as plant height (cm).

vine diameter (mm)
Mucuna flagellipies vine diameter was determine using vernier calipers. The diameter of all the plants in the sampling area of each treatment was measured with vernier calipers "insitu" at 4 cm above the ground. The means was calculated to the nearest millimeter and recorded as plant diameter.

LEAF AREA
Leaf from each *Mucuna flagellipies* plant destructively sampled at 14 WAP and 50% flowering were taken to the laboratory for leaf area determination using Detle-T- Leaf;area meter model (MK-Z).

Leaf Area Index
This was determine by calculation using total leaf area per plant divided by the feeding area of each plant as described by Brown (1984).
LAI = (LA)x (P)-1
Where LAI = leaf Area Index.
LA = Total leaf area per plant
P= ground Area (feeding area of the crop)

Dry Matter (Biomass) weight per plant.
Then destructively sampled plants were separated into fractions (leaf, vine, nodules and roots) and then oven dried to constant weight in forced air oven (hot air typy) at 80^0C for 48 hours for determination of dry matter yield of the plant.

Pod and Seed Yield
Seed yield per plant was determine as a weight of all the seeds of plants harvested from one plot (sampling area) in each treatment and the mean computed to the nearest gram and the score recorded as yield per plant.

Weight per seed
.The weight per seed was measured to the nearest gram and the mean weight of ten randomly selected seeds (of different sizes and weights) was used to compute the score recorded for each treatment.

Statistical Analysis.
All data collected were statistically analyzed using the analysis of variance (ANOVA) procedures as described by Gomez and Gomez (1984). Differences among treatment means were compared using Fishers Least significant difference (F-LSD) at 0.05 probability level (Obi 1986).

RESULTS AND DISCUSSION

The air temperature was warm and fairly uniform at an average of 30.5^0C. While the average soil temperature was 27.4^0C in both 2008 and 2009) seasons (Table 1). Inoculation with *Rhizobium leguminosarum* strains significantly ($P < 0.05$) improved the growth of *Mucuna flagellipies* (Table 2). The number of branches and leaves per plant were higher in all inoculated plants. Throughout the periods under observations (14 weeks after planting (WAP) and 50% flowering), inoculated plants produced taller plants (length of longest vine) with greater leaf area index (LAI) than the non inoculated plants in both 2008 and 2009 seasons. Plants treated with the IAC 636 *Rhizobuim* strains produced plants with the longest vine (235.4 and 238.1 cm at 14WAP) and(361 and 373.2cm) with the highest LAI value of 4.03 and 4.06 at 50% flowering in 2008 and 2009 seasons, respectively. This is in line with the findings of Henzell, (1998) who obtained higher vegetative growth (leaves, stem and branches) in soybeans inoculated with *Rhizobuim* species. The greater number of leaves, branches, vine length and LAI observed in *Mucuna flagellipies* plants treated with *Rhizobium* strains corroborates with report of Kaweski, (1994) who observed significant increases in soybeans plants inoculated with *Rhizobium* strains.

Results showed that *Mucuna flagellipies* plants inoculated with *Rhizobuim* strains produced greater biomass(dry weight of plant parts) than the non inoculated ones (Table 3). At 14 weeks after planting (WAP), leaf and vine dry weight in plants inoculated with CB 188 and CB 756 *Rhizobuim* strains were significantly higher than the control (non inoculated) but did not showed any significant difference between CB 188 and CB 756 *Rhizobium* strains in their leaf and vine dry weight per plant at 50% flowering in both 2008 and 2009 seasons.

On the other hand, all case of inoculation with IAC 636 *Rhizobuim* strains continuously (both at 14 WAP and 50% flowering) produced significantly the highest dry weight of plant fractions than others. Similarly at 50% flowering, the same treatment (IAC 636 Rhizobium strain) produced the highest dry weight per plant; leaf (14.15 and 15.23g) vine (11.32 and 11.44g), nodules (0.78 and 0.79g), root(7.51 and 7.60g) in 2008

and 2009 seasons respectively. This supports the result of Safir *et al.,* (2003) who obtained higher growth and biomass yield of soybeans plants inoculated with *Rhizobium* than the non-inoculated ones. They attributed the higher growth and dry matter yield to the ability of the inoculated plants to fix more nitrogen and improved the soil nutrient status as compared to the non inoculated ones .

Flowering in *Mucuna flagellipies was* significantly improved with *Rhizobuim i*noculation (Table 4). The number of days to first and 50% anthesis was significantly reduced by five to six days in inoculated ones. Similarly, pod formation was earlier in inoculated plants with reduced number of days to first and 50% pod formation. The observed significant improvement in flowering and pod formation in this is in agreement with the work of Okon (1989) who reported similar results where inoculation with *Rhizobuim* resulted in earlier flowering in cowpea than the non inoculated plants.

All plants inoculated with *Rhizobium* strains produced lower numbers and percentage of flowers and pod that aborted per inflorence and per plant, with higher percentage of pod set per plant in the two seasons.

Hunter (2004) reported similar improvement in flowering of cowpea inoculated with *Rhizobuim* inoculants. Additionally, Fawusi, (1999) obtained earlier flowering, pod set and low number of aborted pods in soybeans plants inoculated with *Rhizobuim japonicum*. *Mucuna flagellipies* yield was significantly higher in *Rhizobium* inoculated plant as compared with the non inoculated ones. The number of pods produced per plant were higher in inoculated plants. The best seed yield of 120.2g and 126.1g per plant were obtained in plants treated with IAC 636 *Rhizobium* strains in 2008 and 2009 seasons respectively. Okon, (1989) and Kaweski (1994) had concluded that nitrogen fixation by *Rhizobuim* inoculated legumes gave high yield predominately through it effects on enhanced leaf area index and net assimilation rate. Without *Rhizobium* inoculation, the average *Mucuna flagellipies* seed yield from this study was 79.6 g in 2008 and 82.0g in 2009 were significantly lower than the treated ones. This is in conformity with the result of the work conducted by Fawusi (1999) who recorded lower seed yield in "Ife Brown" variety of cowpea that was not treated with Rhizobium inoculants. He suggested that legumes could be inoculated with appropriate strain of Rhizobium for improve growth and yield.

Table 1: Greenhouse Temperature (Air and Soil) in Polyethelene bags during the Period of the Study (2008 and 2009 Seasons).

Months	Temperature ^{0}c. Soil in poly bags	Air
2008 Season.		
April	29.5	35.5
May	29.3	
June	28.1	32.5
July	26.5	31.8
August	27.4	30.9
September	26.5	32.5
October	26.3	31.7
November	27.2	30.3
December	25.3	29.7
2009 Season		
April	29.3	35.4
May	29.2	33.1
June	27.5	32.3
July	26.6	31.4
August	27.1	30.5
September	26.6	31.4
October	26.7	30.3
November	26.7	30.1
December	25.7	29.6

Table 2: Effect of Rhizobium Inoculation on the Vegetative Growth of Mucuna Flagellipies during 2008 and 2009 Cropping Seasons in the Greenhouse.

Treatments Rhizobuim Strains	**14 Weeks After Planting Vegetative growth attributes**					**50% flowering Vegetative growth attribute**					
2008 Seasons	No. of leaves per plant	No. of Branches per plant	Longest vine length (cm)	Vine Diameter (mm)	Leaf Area index (LAI)	No. of leaves per plant	No. of Branches per plant	Vine Diameter (mm)	Leaf Area index (LAI)	Longest Vine length (cm)	LSD(0.05)
2008 season											
No Rhizobium strain (control)	12.2	2.2	194.4	22.0	0.16	20.1	4.1	38.2	1.57	263.3	0.54
CB 188	16.3	2.5	210.7	30.8	1.02	26.3	6.1	47.5	3.79	324.1	1.25
CB 756	16.5	2.6	218.0	30.9	1.15	27.9	6.3	48.7	3.81	328.4	1.25
IAC 636	18.4	2.7	225.5	34.4	1.33	30.2	7.5	61.8	4.03	361.9	2.35
LSD (0.05)	1.2	NS	13.5	1.1	0.01	2.0	0.3	4.4	0.3	28.5	0.11
2009 Season.											
No Rhizobium strain (control)	12.5	2.2	196.7	23.9	0.17	21.1	4.1	39.1	1.68	265.4	1.55
CB 188	17.2	2.4	214.4	31.5	1.06	28.3	6.2	49.3	3.83	328.3	1.26
CB 756	17.3	2.6	220.2	31.7	1.07	28.5	6.5	49.6	3.87	329.5	1.26
IAC 636	19.1	2.7	228.1	36.6	1.35	32.3	7.7	66.4	4.06	373.2	2.37
LSD (0.05)	1.2	NS	18.7	1.2	0.01	2.1.	0.3	4.5	0.3	28.5	0.11

Table 3: Effects of Rhizobuim Inoculation on Growth (biomass dry weight (g) of *Mucuna flagellipies* in the green house during 2008 and 2009 Seasons.

Treatments Rhizobuim Strains	**14 Weeks After Planting**				**50% flowering**				
	Biomass, Dry weight of plant fraction				**Biomass, Dry weight of plant fractions.**				
	Leaf dry weight per plant (g)	Vine dry weight (g)	Nodule dry weight per plant (g)	Root dry weight per plant (g)	Leaf dry weight per plant (g)	Vine dry weight per plant (g)	Nodules dry weight per plant (g)	Root dry weight per plant (g)	LSD (0.05)
2008 Season									
No Rhizobium strain (control)	2.41	3.62	0.06	0.23	9.54	5.14	0.30	4.46	0.12
CB 188	3.46	4.35	0.11	0.29	13.19	8.83	0.44	6.32	0.01
CB 756	3.37	4.39	0.13	0.34	13.42	9.20	0.74	6.32	0.01
IAC 636	4.11	5.51	0.20	0.37	14.15	11.32	0.78	7.51	0.31
LSD (0.05)	0.2	0.3	0.01	0.03	0.78	1.13	0.03	0.2	0.001
2009 Season									
No Rhizobium strain (control)	2.36	3.38	0.06	0.24	9.50	5.15	0.31	4.47	0.12
CB 188	3.29	4.21	0.12	0.31	13.17	8.85	0.46	6.15	0.01
CB 756	3.67	4.57	0.14	0.35	14.52	9.20	0.60	6.39	0.01
IAC 636	5.12	5.63	0.21	0.41	15.23	11.44	0.79	7.60	0.33
LSD (0.05)	0.2	0.3	0.01	0.03	0.77	1.04	0.03	0.2	0.001

Table 4: Effects of Rhizobium Inoculation on Flower and pod Formation in *Mucuna flagellipies* in the green house during 2008 and 2009 seasons.

Treatments *Rhizobuim* Strains	**Flower formation**					**Pod formation**					
2008 Seasons	Days to first flowering	Days to 50% flowering	No. of flower per inflorence	No. of flowers aborted per inflorence	Percentage of flowers aborted per inflorence	Days to first pod formation	Days to 50% pod formation	No. of pod aborted per plant	Percentage of pods aborted per plants	Percentage of pod set per plant.	LSD (0.05)
No Rhizobium strain (control)	168.3	182.4	7.5	6.3	84.0	185.8	192.6	7.3	61.9	38.1	6.1
CB 188	163.8	178.5	8.3	4.5	54.2	181.5	187.6	5.1	23.3	66.7	3.2
CB 756	150.0	175.0	8.1	4.5	55.6	179.5	185.2	4.3	23.5	66.5	3.2
IAC 636	153.1	172.3	7.5	2.3	30.7	175.3	181.6	4.5	22.9	78.1	4.1
LSD (0.05)	2.4	1.5	NS	0.3	13.5	3.1	2.3	0.5	5.3	15.7	2.4
2009 seasons.											
No Rhizobium strain (control)	169.2	182.2	8.1	6.5	80.2	192.6	193.9	7.5	60.9	39.1	6.1
CB 188	166.5	178.9	9.5	4.5	49.4	187.6	188.6	5.2	32.5	67.5	3.2
CB 756	160.9	175.9	8.5	3.5	41.2	185.2	184.2	4.1	22.1	77.9	3.2
IAC 636	156.1	173.6	8.3	3.2	38.6	181.6	18.5	4.3	21.9	78.1	4.2
LSD (0.05)	2.4	1.5	NS	0.3	14.3	3.1	2.3	0.5	6.2	16.8	2.5

Table 5: Effects of Rhizobuim Inoculation on yield of Mucuna flagellipies in the green house during 2008 and 2009 Seasons.

Treatments Rhizobuim Strains	Pod yield per plant (g)	Seed yield per plant (g)	Weight per Seed (g)	LSD(0.05)
2008 season				
No Rhizobium strain (control)	132.1	79.6	4.5	0.5
CB 188	169.1	95.8	15.2	0.3
CB 756	188.1	108.1	16.2	0.3
IAC 636	214.2	120.2	18.2	0.4
LSD (0.05)	17.5	12.2	1.7	0.1
2009 season				
No Rhizobium strain (control)	136.1	82.0	5.6	0.5
CB 188	176.7	101.8	16.3	0.3
CB 756	189.3	114.3	17.2	0.3
IAC 636	228.3	126.1	18.5	0.4
LSD (0.05)	18.5	11.5	5.6	0.1

CONCLUSION AND RECOMMENDATION

From the above results, it could be concluded that yield advantages were gained by inoculation of *Mucuna flagellipies* with some strains of *Rhizobium leguminosarum.* Therefore, we recommend that further studies with these strains of *Rhizobuim leguminosarum* should be carried out in the field to evaluate their interactions naturally with the indigenous soil *Rhizobuim* species and the native soil micro flora /fauna. *Mucuna flagellipies* should be inoculated with IAC 636 Rhizobium strain for optimum growth and yield also could be use as biofertilizer an alternative to mineral nitrogen fertilizer.

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