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SUSTAINABILITY OF ENDOGENOUS FARMING PRACTICES IN CONTROLLING PLANTS DISEASES: CASE OF SEEDING DENSITY ON *MACROPHOMINA PHASEOLINA*

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A B S T R A C T

In Niger Republic, one of the endogenous farming practices adopted by small-scale farmers to control severe damping-off and improve the plant stands of cowpea (*Vigna unguiculata* L., Walp) is high seeding density. This study aims to evaluate the effect of this practice in controlling the charcoal rot of cowpea caused by *Macrophomina phaseolina*. The field experiment was conducted at AGRHYMET Regional Centre of Niamey consisted of; plots sown at a density of 1, 2, 3, and 4 seeds per planting hole. The results showed that 10 days after sowing, the plant stand was significantly higher in plots sown with one seed per planting hole (83%) whilst the plots sown with 3 and 4 seeds per planting hole had the lowest plant stand (63%). The plots with seeding densities 4 and 3 seeds per planting hole recorded the highest incidence of the disease at the harvest period with 44% and 33% of dead plants respectively. Furthermore, in the plots sown with 4 seeds per planting hole, the *density* of *M. phaseolina* per root tissues was the highest (21680 sclerotia.g^{-1}). The seeding density did affect also the pod and average grain yield of cowpea, as compared with high seeding densities plots. According to the results of the study, the small-scale farmer's endogenous farming practices of varying seeding density increase the severity of charcoal rot and reduce yield. Consequently, the agricultural services should raise the awareness of the small-scale farmers about the threat of such practices to the development of the cowpea sector.

Keywords: *Macrophomina phaseolina,* seeding density, soil inoculum, endogenous farming practices, sustainability.

INTRODUCTION

Worldwide, the Sub-African is one of the largest regions of cowpea production (Alemu *et al*., 2016). The cowpea production in this region is estimated at 6.2 million tons for a cultivated area of 10684706 ha. Nigeria is the main word cowpea producer country with 83.5% of the production, followed by Niger (1.96 million tons). Burkina Faso, Mali, and Senegal are other cowpea producers of less importance in Africa (FAO, 2018). West Africa populations are the main cowpea consumers especially in the rural areas where cowpea constitutes an important source of protein (FAO, 2018).

However, several constraints affect cowpea production, including pests and diseases, low soil fertility, drought, lack of inappropriate cultivars and lack of agricultural inputs (Ajeigbe *et al*., 2010). One of the most threatening disease in cowpea cultivation is *M. phaseolina*, the causal agent of ashy stem blight. This pathogen causes pre, post-damping off, and withering of seedlings and mature plants. These symptoms on cowpea are the result of the necrosis of roots, stems and mechanical plugging of xylem vessels by microsclerotia, produce phytotoxins such as phaseolinone and botryodiplodin (Chan and Sackston, 1973; Ramezani, 2008). One of the most obvious symptoms include a rapid wilting and drying of the whole plant, the presence of black bodies on the stem and the branches of the plants (microsclerotia) that resulted in the appearance of charcoal or ashy of the

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dead plants (Short *et al*., 1980). The microsclerotia are the survival form of the pathogen in the soil and commonly their soil density is inversely correlated to the cowpea yield. *M. phaseolina* is a polyphagous soil-borne pathogen (Su *et al*., 2001) that can be hosted by more than 500 cultivated and wild plant species. In addition, the fungus can survive in dry soil for more than three years or by colonizing and producing microsclerotia in dead plant tissues in a saprophytic way (Ndiaye *et al*., 2008).

Many studies have highlighted various methods in controlling *M. phaseolina*. However, chemical control of *M. phaseolina* is not feasible due to the saprophytic nature and prolonged survival nature of the pathogen (Dave *et al*., 2021). The same authors indicate that the chemical fungicides are expensive, toxic and hazardous and sources of pollution. However, many researchers indicated an alternative approach to control the damages caused by *M. phaseolina.* A combination of weeds and mustard residues, actinomycetes, lytic bacterial density has shown a microbial antagonism to *M. phaseolina (*Sharma *et al*., 1995; Mawar and Lodha, 2002; Israel *et al*., 2005). In addition to the methods of tillage and crop rotation, cowpea producers also use biological control as a way to counter the impacts of *Macrophomina phaseolina* (Ndiaye *et al.*, 2010; Dave *et al.*, 2021). Fluorescent Pseudomonas, such as *Pseudomonas psychrotolerans* has antagonistic activity against *M. phaseolina* (Manjunatha *et al*., 2012; Muhammad *et al.,* 2015).

The management of plant diseases is an important condition for the development of sustainable agriculture (Hamed *et al.,* 2018). Commonly, in the Republic of Niger small-scale farmers produced cowpea in a monoculture system or mixed with other crops such as millet or sorghum. These small-scale farmers often lack good quality seeds and resistant varieties of cowpea to pests and diseases. Recently, it is observed that they are increasing the number of seeds per planting hole (Ndiaye *et al*., 2007) to prevent severe damping-off of the plants and improve the plant stands. Such endogenous farming practice could compromise the development of crops, susceptible to *M. phaseolina*, due to the rapid inoculum building up and long conservation of the soil inoculum of the pathogen. Therefore, this study aims to evaluate the effect of increasing seeding density to control the charcoal rot of cowpea in rural areas of the Republic of Niger. Especially, the study seeks to examine how sustainable is such practice in controlling the charcoal rot by examining relationships between; (1) the seeding densities per planting hole and the plant stand, (2) the seeding densities per planting hole and the disease incidence, (3) the seeding densities per planting hole and the pod and grain yields and (4) the seeding densities per planting hole and the potential inoculum of sclerotia per gram of root tissues.

MATERIALS AND METHODS

Materials: Seed material: The seeds of the cowpea cv. Mouride were obtained from the National Agricultural Research Institute of Senegal (ISRA) and multiplied at AGRHYMET Centre for the experiment. Mouride is resistant to bacterial canker, *Striga gesnoroides*, and cowpea aphid-borne mosaic virus and is widely cultivated in Guinea Bissau, Niger, Chad, and Ghana (Hall *et al.*, 2003). Mouride has been used in this study to minimize the effect of co-infection, mainly with Striga. The seeds did not undergo any sanitary treatment just like farmers.

Field trial site and experimental design: Field trial plots were located at the Regional Centre AGRHYMET between 13°28 and 13°35 North latitude and 2°03 and 2° 10'' East longitude in Niamey, Niger Republic. The experimental site is characterized by a long dry season (November-May) and a rainy season (June-October) and tropical ferruginous soil with a predominance of sandy soil.

The experiment was carried out in naturally infested soil by *M. phaseolina* in a complete randomized block design. Four different densities 1, 2, 3, and 4 seeds per planting hole- were tested in 4 blocks of 348.5 m^2. The blocks were separated by 1.5 m and the elementary plots by 1 m. Accordingly; each elementary plot contained 5 lines of 8 planting holes and a distance of 0.5 m from each other. The mean temperature was 31°C, the relative humidity was 56% and a total rainfall of 456 mm was recorded during the experiment period (June 2013 – September 2013).

Methods: Soil sampling and analysis for *Macrophomina phaseolina* inoculum estimation: To estimate the density and the distribution of initial inoculum in the experimental plots, a sample of 500 g soil was collected from each elementary plot at 15 cm deep with an auger accordingly to the "W" method. The collected samples were dried in the laboratory under ambient conditions for 7 days and then were analyzed in a semi-selected medium for *M. phaseolina* (Ndiaye *et al.,* 2007). The number of colonies of *M. phaseolina* developed in the Petri dishes was counted 10 days after incubation.

Plant stand, disease incidence and yield: The Plant stand, the disease incidence, and yield were determined from the 3 central lines of each experimental plot. The plant stand was determined as the proportion of seedlings 2 weeks after planting. The disease incidence, the percentage of dead or heavily withered plants per plot were recorded weekly for 10 weeks and the rate of the disease development (slope lines of the disease progress curves) was estimated by the linear regression analysis. In addition, Shaner and Finney, (1977) formula was used to determine the standardized area under the disease progress curve (AUDPC).

$$\text{AUDPC} = \sum_{i=1}^{n} \frac{(Xi + Xi - 1)}{2x(ti - ti - 1)}$$

Where n is the number of evaluation times, x_i the incidence of the disease per evaluation. (x_{i-1}) the disease incidence at the previous evaluation time and (t_i–t_{i-1}) the time duration.

At the maturity stage, mature pods were picked regularly, dried at the ambient temperature, pooled and then weighted before and after threshing.

Root tissue analysis for microsclerotia of *Macrophomina phaseolina:* To determine the potential telluric inoculum, plant roots were collected at harvest from each plot, dried in the laboratory under ambient conditions, cut off in 5 mm pieces and grinded in a Mixer (Mikro Feinmühler Cullati. Modèle DCFH 48 (IKA Labortechnick)). 25 mg of the grinded root tissues were plated in a semi-selective medium for *M. phaseolina*, incubated at 30 °C and the number of colonies of *M. Phaseolina* developed in the Petri dishes counted after 10 days.

DATA ANALYSIS

Genstat program for Windows 12th Edition (IACR-Rothamsted. the UK) was used for data analysis. All the data were subjected to analysis of variance (ANOVA) following a randomized complete block design. The treatment means were compared using Duncan's Multiple Range Test at P = 0.05. Where needed data were transformed by log (x+1) before statistical analysis.

RESULTS

Effect of seeding density on plant stand, charcoal rot disease incidence and yield of cowpea: The initial soil inoculum of *Macrophomina phaseolina* was low (6 microsclerotia/g soil) and statistically equal in all treatment plots (P>0.05). Among the tested seeding density, 1 seed per planting hole produced the highest plant stand (83%) and the lowest disease incidence (14%), 2 weeks after planting. However, there were no significant difference between treatments 2, 3 and 4 seeds per planting hole for the plant stands, and 1 and 2 for disease incidence (Table 1). There was significantly more pod and yield grain (1192 kg and 861 kg pods and grain, respectively) in the plots planted with 1 seed per planting hole than in the plots planted with 2, 3, or 4 seeds per planting hole. However, the smallest yields and the highest density of sclerotia per gram roots (potential inoculum) were recorded in plots planted with 4 seeds per planting hole (Table 1).

Table 1. Soil inoculum plant stand, disease incidence, and yields in plots planted with 1, 2, 3, and 4 seeds per planting hole.

Seeds per planting hole (hole)	Plant stand (%)	Disease incidence (%)	Pod yield (kg/ha)	Grain yield (kg/ha)	Potential inoculum (# of sclerotia/g root tissues
1	83.33^{a}	13.76^{b}	1192.0^{c}	861.2^{C}	810^{a}
2	65.10^{b}	20.04^{b}	1000.5^{b}	774.8^{bc}	995^{b}
3	63.01^{b}	33.69^{a}	963.2^{b}	742.4^{b}	24981^{c}
4	63.02^{b}	43.54^{a}	753.7^{a}	582.2^{a}	216802^{d}

For each column, the means followed by the same letters are statistically equal

Relationship between seeding density, yields of cowpea and inoculum production of *Macrophomina phaseolina*: Table 3 presents the results of correlation analysis between seeding density, plant stand, harvested plant, grain yield per plant, pod yield per plant and sclerotia per gram of root tissues at harvest. Seed density was highly and positively correlated with plant stand, harvested plants and root sclerotia per gram tissue, but negatively related to pod and grain yields (-0.919 and -0.930 respectively). There were strong positive relationships between the number of sclerotia per gram tissue and the plant stand (0.830), harvested plants (0.859) and grain yield (0.785), but merely negatively related with pod yield (-0.617). There was no relationship between the density of sclerotia per gram of root tissues and cowpea grain yield (0.268).

Table 3. Relationship between seeding density, plant stand, harvested plants, grain and pod yields of cowpea per plant, and density of sclerotia per gram of root tissues

Seeding density	1	1					
Plant stand	2	0.998	1				
Harvested plants	3	0.996	0.988	1			
Grain yield per plant	4	-0.919	-0.891	-0.949	1		
Pod yield per plant	5	-0.930	-0.903	-0.958	0.530	1	
Sclerotia per gram of root tissues	6	0.830	0.859	0.785	0.268	-0.617	1
		1	2	3	4	5	6

Effect of seeding density on the Disease Progression Curve (DPC) of charcoal rot of cowpea: The disease development rate was low and similar from day 21 to day 63 for sph 2, 3, 4, but higher than for sph 1. From day 63 onward, however, the rates of the disease progression curves were high and significantly different in all treatments, but planting one seed per planting hole resulted in less disease and lower disease progression rate during the whole growing period (Figure 1 and Table 4).

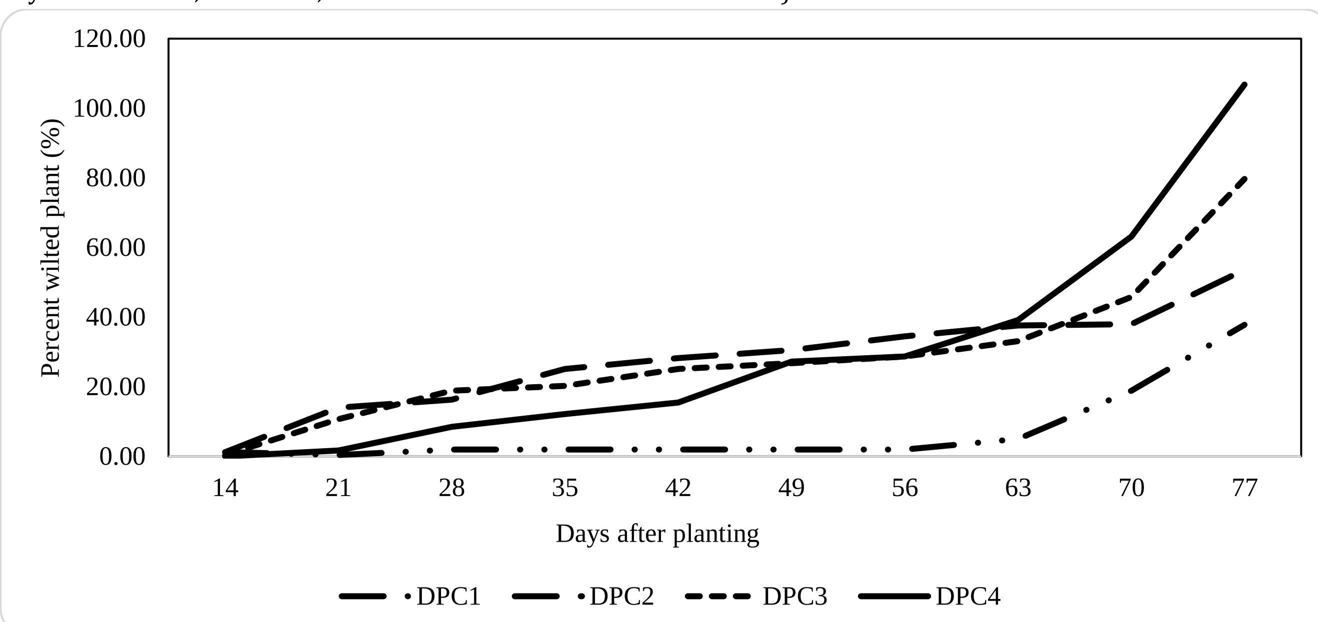

Figure 1. Disease progress curves (DPC) of charcoal rot of cowpea in the field naturally infested with *M. sp.* and planted with 4 seeding densities (1, 2, 3, and 4 seeds per planting hole (sph)). The disease development was assessed weekly for 10 weeks after planting.

There was a significant difference observed among the rate parameters of the linear regression model in seed planting densities (P< 0.001). Seed planting density 4 had the largest Y_0r and highest r (0.015 CFU per day) while sph 1 had the lowest Y_0r and r (0.005 and 0.006 CFU respectively) (Tableau 4).

Table 4. Linear regression of logit-transformed disease data against time for four seed planting densities of cowpea in a field naturally infected by *Macrophomina phaseolina*

Number of seeds per planting hole (sph)	Y_0	Y_0r	r	R^2	Standard error
1	5.00	0.005	0.001	0.53	0.002
2	5.90	0.006	0.001	0.92	0.001
3	5.10	0.009	0.002	0.80	0.002

4	4.65	0.015	0.003	0.84	0.003

Y_0 = Initial inoculum; Y_0r = slope of the line estimated by the regression equation; r = Rate of inoculum production per day

Area under the Disease Progression Curve (AUDPC: The AUDPC (Figure 2) was significantly lower (P<0.001) for the treatment sph 1 (138) and sph 2 (189) compared to sph 3 (466) and sph 4 (877). However, the AUDPC of sph 3 was significantly higher than sph 1 and sph 2, but significantly lower than those of sph 4 (Figure 2).

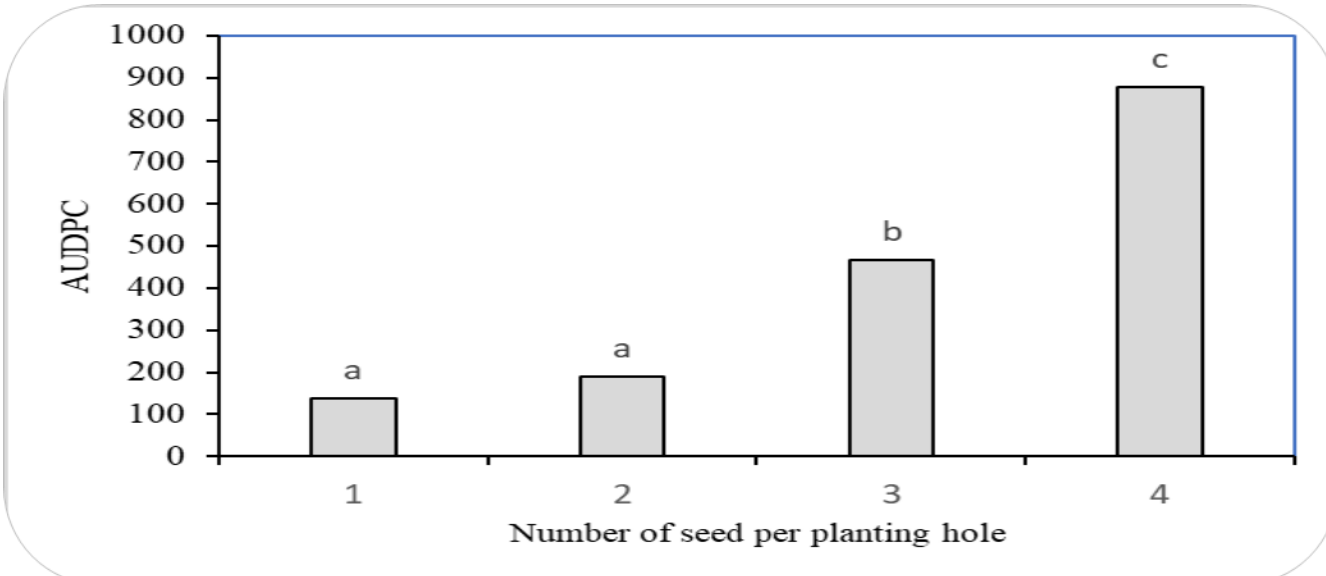

Figure 3. The area under the disease progression curve of the number of seeds per planting hole

DISCUSSION

In Niger, field cowpea is often severely affected by damping off due to disease or dry spells at the beginning of the rainy season. Macrophomina phaseolina, a seed-borne, soil and seed source pathogen that causes charcoal rot is the most widespread disease in Sahel regions and the Niger Republic, causing seedling deaths and yield loss (Ndiaye, 2007). Commonly, the famers/ growers of cowpea in the Niger Republic increase the number of the seed of cowpea seeds per planting hole to control the damping-off and optimize the plant stand of the cowpea. The current study evaluates the effect of this practice in controlling the charcoal rot of cowpea caused by *M. phaseolina.*

The results showed that seeding density affected the plant stand, disease incidence, pood and grain yields of cowpea. Accordingly, the seedling density 1 seed per planting hole recorded the highest plant stand, the lowest disease incidence and the lowest density of sclerotia per gram of root tissues compared to the seedling densities 2, 3 and 4 seeds per planting hole. It might be that the lowest rate of plant stand, the highest disease incidence and density of sclerotia per gram of root tissue in the seedling holes 2, 3, and 4 are due to the progression of the infection from one plant root to another within the planting hole of these seedling densities. This result is similar to that of Burdon and Chilvers, (1975) who showed that the seedling density and damping-off disease *Pythium irregulare*) in *Lepidium sativum* resulted from the transmission of pathogens from hosts to others. Besides, the highest disease incidence and density of sclerotia per gram of root tissue in seedling holes 2, 3, and 4 seeds could be due to the presence of sclerotia in cowpea seeds as the primary source of the disease. Similarly, Ndiaye, (2007) and Luna *et al*. (2017) have previously found that the mycelium of M. phaseolina in the seeds and plants residues and sclerotia in the soil serve as one of the main sources and vectors in spreading charcoal root rot. In addition, Short *et al*. (1980) noted that the initial density of sclerotia in the soil is directly correlated with the severity of charcoal rot of soybean.

A correlation analysis of the seeding densities with cowpea yields showed strong relationships between plant stand, harvested plant, and sclerotia density per gram of root tissue. This result aligned with those found by Kaur *et al*. (2012) who mentioned that under some conditions *M.* phaseolina cause important yield losses in numerous crops such as cowpea. Furthermore, it was observed that for a density of 1 seed per planting hole, the number of sclerotia formed in roots was 10, 100, and 1000 times smaller than that of 2, 3, and 4 seeds per planting hole, respectively. This may explain the lowest slope (Y0r) and rate of inoculum production per day (r) of the 1 seed per planting hole revealed by the linear regression model of the density-response data. This increase of the rate of daily inoculum production of M. phaseolina might spread the disease easily on the plants and therefore result in accumulating density of sclerotia in the root tissues at harvest.

AUDPC was constructed to reflect all aspects of disease progression related to the growth and development of the host as described by Royle (1994). AUDPC results showed a significant link between yield production and charcoal development as affected by seeding density. Besides, it was found that the higher AUDPC, Y_0r and r, the lower yields recorded by 3 and 4 seeds per planting hole plots. Also, interspecific competition and synergy with disease effect could lead to a loss of productivity per plant as the increase in seedling density increases the number of plants per unit area that compete for limited resources (water, nutrients, light).

CONCLUSION AND RECOMMENDATION

This study evaluates the effect of increasing the number of seeds per planting hole as an endogenous method in controlling the charcoal rot of cowpea caused by *Macrophomina phaseolina*. The results of the study showed that practice increases the daily production rate of inoculum and the accumulating of the sclerotia of *M. phaseolina* in the root tissues of the plant at harvest time as well reducing the pod and grain yields of the cowpea. Consequently, the endogenous farming practice consisting of increasing the number of seeds per planting hole to control *M*. *phaseolina* are not a sustainable one and would result in in increasing the soil density inoculum and loss of yields. This would threaten the development of cowpea and all other host crops of *M. phaseolina*. Consequently, agriculture-related institutions and other agricultural extensions services should raise the awareness of the small-scale farmers about the threat of their current practices to the development of the cowpea sector.

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Hamidou T. A. Azize	:	Designed and conducted the experiment, recorded and analyzed the study data, and drafted the manuscript under the supervision of Dr. Mbaye NDIAYE.
Mbaye Ndiaye	:	Revised the data record and analysis, and drafted the manuscript as well as edited the final version of the manuscript before its submission to the journal.