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Research Article

Effects of Seed Coating Agents on Some Agronomic Properties and Rhizobacterial Population of *Vigna unguiculata*

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Abstract
The agricultural activities had tremendously benefitted from the application of pesticides. However, various objectionable practices among farmers had exposed the soils, plant and its community of organisms to different level of toxicity. Therefore, this study evaluated the effects of seed coating agents on germination rate, shoot length, leave numbers and population of rhizobial and phosphate solubilizing bacteria (PSB) of beans plant (*Vigna unguiculata*). The mesocosm experiment was carried out in a greenhouse using a randomized block design with five replicates over the period of 5 weeks. The seed coating agents used were Imidadoprid-Thiram powder formulations and black powdery waste from dry-cell batteries. The seed coating agents were applied at the recommended rate value of 1.25 g/kg and at the rate that double the concentration of recommended field rate (2.50 g/kg). The application of Imidadoprid-Thiram at recommended rate and spent battery waste at the same rate did not have any significant effect ($p > 0.05$) on the germination of *V. unguiculata* as compared to the control. However, significant reductions ($p < 0.05$) in germination were observed in the seed coated with both coating agents at the rate above the recommendation value. Likewise, no significant effect ($p > 0.05$) of seed coating agents was observed on the average number of leaves of growing *V. unguiculata* seedling but significant reductions ($p < 0.05$) were observed in the shoot length of all the treated seedling as compared with control. Both Imidadoprid-Thiram and spent battery wastes were observed to have a significant negative ($p < 0.05$) effect on the average numbers of both rhizobia and PSB counts in the rhizosphere region of *V. unguiculata*, with effects more pronounced in higher doses.

Keywords: *Agronomic properties, Imidadoprid-Thiram, Phosphate solubilizing bacteria, Rhizobia, Vigna unguiculata*

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Introduction

Successful management of agro-ecosystem of crop land is majorly relied on the discovery and application of effective pest control measures (Cooper and Dobson, 2007). Among this is the use of synthetic pesticide and other toxic agents. Pesticides are important agrochemicals that are widely use to boost agricultural activities. However, due to high cost of pesticide procurement, farmers had devised other means as an alternative for pest management (Ajayi, 2005). Among these is the use of Manganese-IV-oxide containing black colour and powdery by-product of spent battery. In rural area of Nigeria, treatment of seed with dry spent battery waste as alternative to recommended seed coating pesticide is a common practice because of its effectiveness in protecting the crop during its pre-emergence stage (Ikhajiagbe and Guobadia, 2016). In developing countries, the use of dry battery waste in controlling termite is a common practice (Peprah, 2014). Imidadoprid-Thiram formulations are neonicotinoid systemic insecticides used to control insects and find wide application in topical use on pests, crops, soil, and as a seed treatment agent (Shakir *et al*., 2016).

Seed treatment procedures involve the application

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of chemical substances to the seed prior the period of sowing. These substances could be synthetic chemical substances or naturally occurring compounds. In some cases, beneficial microbes could be used in the treatment of seeds prior to sowing. Seed treatment technique has been reported to provide competitive advantages over other methods because of its precision in getting the products into the field, and its characteristic high level of effectiveness against pest (Sharma *et al*., 2015). Likewise, the potential of minimizing the possible exposure of humans and other environmental components to agrochemical products is one of the competitive advantages over other methods. These treatments aid in suppressing, repressing and controlling the pathogens and other pests that could attack seeds and growing plants. Hazra and Patanjali (2016) reiterated that seed treatment technique was unique in that it offers protection against plant pests and pathogens especially during the susceptible period of development. Also, seed treatment technique was confirmed to be crop-responsive, grower friendly as well as environmental safe (Irfan *et al*., 2020).

Soil is a unique medium of bio-interactions. The indiscriminate use of pesticides could disturb the soil environment by affecting flora and fauna including microflora of soil (Ram *et al*., 2020). Soil microbiota has been reported to be the key engine that drives the biotransformation processes in the soil system (Gougoulias *et al*., 2014). The chief among them include those that have direct link to plant productivity such as phosphate solubilizing bacteria (*Pseudomonas* spp., *Bacillus* spp. and *Rhizobium* spp), fungi (*Aspergillus* spp. and *Penicillum* spp.) and actinomycetes (Kalayu, 2019). Nitrogen fixing bacteria that colonize the rhizospheric region of growing plant are also known to play some major roles in plant survival and productivity (Bano and Iqbal, 2016).

Pest management and control has been a key step in boosting agriculture productivity; thus stabilization and improvement of beneficial microbial communities within the rhizosphere region might be important factors for achieving a sustainable agriculture. Generally, rhizosphere microbes are known to play some unique roles in plant productivity through their activities such as biotransformation of nutrients, growth enhancement and diseases suppression (Fankem *et al*., 2008; Odelade and Babalola, 2019). *Vigna unguiculata* commonly called cowpea is one of the most popularly cultivated grains in Africa (Kyei-Boahen *et al*., 2017). It is a leguminous plant that benefitted directly from symbiotic interactions which exits between its root regions and rhizosphere microbiome in the soil system (Igiehon and Babalola, 2018).

Ecologically, the disproportionate introduction of chemical into the environment could undesirably disrupt the ecological balance and even affect the plant-supportive functions of the rhizospheric microbiota and thereby affect ecological sustainability (Hazra *et al*., 2017; Roberts and Mattoo, 2018). The objectionable practices that involve soil management and introduction of pesticide among farmers could expose soils, plant and its community of organisms to different level of toxicity. Among such practices, are the applications of pesticide above recommended dose and the use of unregistered toxic products especially, in developing countries (Shakir *et al*., 2016). Therefore, this study evaluate the effect of seed coating agents on agronomic properties (germination rate, shoot length and leave numbers) and population of some selected beneficial rhizobacterial (rhizobial and phosphate solubilising bacteria) of *Vigna unguiculata*.

Materials and Methods

The study area

The study was conducted at the green house facility of Kwara State University Teaching and Research Farm, Malete, Nigeria. The coordinate of the study area is within the latitude 08^{o} 43N and longitude 04^{o} 19E. The area is characterized by bimodal patterns of rainfall from April to July, then from September to November with short spell in August. The soil used for planting was collected from Kwara State University Teaching and Research Farm, Malete, Nigeria and classified as Plinthosol type with the following average agrochemical features; 29.3 % of total organic carbon, 9.6 g/kg phosphorous content, 18.3 % moisture content and pH_{H20} value of 7.1 (Alabi *et al*., 2017).

Experimental design

The experiment was set-up by adopting a microcosm random block design with five replicates to assess the impact of seed coating chemical on growth parameter of beans seeds and some selected group of beneficial rhizosphere bacteria of leguminous plant. The seed coating agents used were Imidadoprid-Thiram powder formulations (an approved insecticidal seed dressing agent) and black powdery waste from dry-cell batteries which is commonly used among the local farmers in rural area of Nigeria.

The experimental material used for this study was beans of the genus *Vigna unguiculata* (L.) Walp and purchased from a certified agro-products vendo (MATTO AGRO AND CHEMICALS), in Ilorin. The seeds were subjected to viability test prior to treatments and sowing. About 1.25 g of seed treatment agents per 1 kg of beans seed as recommended for Imidadoprid-Thiram powder formulations by manufacturer was adopted for both coating agents in the treatment of beans seed before sowing.

The five treatment groups used for this experiment were: "IT": 1.25g of Imidadoprid-Thiram to 1 kg of bean seed; "2IT": 2.50g of Imidadoprid-Thiram to 1 kg of bean seed; "BW": 1.25g of Battery waste to 1 kg of bean seed; "BW": 2.50g of Battery waste to 1 kg of bean seed; "UT": (control), untreated seeds.
Each treatment was thoroughly mixed in its allotted bucket at the beginning of the experiment.

The effect of the coating agents was determined on the germination rate, shoot length and number of leaves of *V. unguiculata*. The germination rate was observed over the period of 6 days. Also, soil samples from rhizosphere region of each experimental unit were collected every week for a period 5 weeks to assess the impact of coating agents on the culturable rhizobia and phosphate solubilizing bacterial communities of *V. unguiculata* seedlings.

Evaluating the effects of seed coating agents on seed germination and seedling growth of Vigna unguiculata

The effect of treatments on seed germination was evaluated following the standard testing method of International Seed Testing Association as described by Defang *et al*. (2010) with slight modification. Ten (10) viable seeds from each treatment group were randomly planted in about 500 cubic centimeters capacity nursery bags and covered with soil. The moisture contents of each experimental unit were maintained at about 80 % by spraying evenly with distilled water.
Seed was considered germinated after emergence of the radicle and germination percentages were calculated using the following formula:
"PG" = (NGS_p/ TPS) x 100"

Where;
"PG" represents percentage germination rate (%); "NGS" represents the number of germinated seeds; p is the period of observation; and TPS is the total number of planted seed per each experimental unit

Seedlings were thinned to three in each experimental unit to further observe the effect of seed coating agents (Imidadoprid-Thiram and battery waste) on other agronomic parameters of growing seedling at 1 week interval over the period of 5 weeks after planting.

Estimation of rhizobia and phosphate solubilizing bacterial population in rhizosphere region of growing Vigna unguiculata seedlings

One gram of soil sample from the rhizosphere region of growing *V. unguiculata* seedlings of each experimental unit comprises of 5 replicates was collected and properly mixed together to make a composite sample. A suspension of 1 g of the composite sample in 9 ml distilled water was prepared and further diluted serially (ten-fold). About 0.1 ml of 10^7 diluted concentrations was inoculated onto solid agar media using pour plate method in three replicates. The inoculated plates were inverted and incubated at room temperature (25 ± 2^o C) for 48 hours before enumeration by counting the visible colonies formed.
The media used for the estimation of rhizobia and phosphate solubilizing bacterial groupswere Congo-Red-Yeast-extract-Mannito agar and Pikovskaya's solid medium respectively.

Data analysis

The data collected on agronomic properties of *Vigna unguiculata*, rhizobia and phosphate solubilizing bacterial population were subjected to

statistical analysis and represents as the mean ($\bar{x}$) of the total values. The variability of the data was expressed in terms of their standard error (σ) of mean value. The data were further subjected to Analysis of Variance (ANOVA) and the differences among the mean values were established using the Least Significant Difference (LSD) test at significant level of 5%. Analyses were carried out on IBM-SPSS version 23and Statistical Tool for Agricultural Research (STAR).

Results and Discussion

Impact of seed coating agents on germination rate and other agronomic parameters of Vigna unguiculata

The percentage germination values (%) for the treated *V. unguiculata* seeds are presented in Table 1. The emergences of the *V. unguiculata* plumules were first observed at the 3rd day after planting in all the treatments (Table 1). At the 3rd day after planting, the germination rate (%) observed were 48, 22, 52, 28 and 67 for the treatments IT, 2IT, BW, 2BW and UT respectively. The germination rate values (%) at the 4th days after planting were 64, 44, 72, 46 and 72 for the treatments IT, 2IT, BW, 2BW and UT respectively. At the 5th rate after planting, the germination (%) values were; 92, 72, 88, 72 and 98 for the treatments IT, 2IT, BW, 2BW and UT respectively. At the 6th day after planting, 100 % germination rates were observed for untreated cowpea seeds (UT) and the seed treated with recommended rate of Imidadoprid-Thiram (IT). However, the results on the effects of seed coating agents on *V. unguiculata* showed that the application of Imidadoprid-Thiram at recommended rate and the spent battery waste at the same rate did not have any significant effect ($p > 0.05$) on the germination of the experimental plant as compared to the control. However, significant reductions ($p < 0.05$) in germination were observed in the seed coated with coating agents at the rate above the recommendation value. This finding was in accordance with the report of Aliero *et al*. (2014), which revealed that the application of butachlor and pendimethalin pesticides at concentration above 5 mg/ml significantly inhibited the germination rate of *V. unguiculata* as well as *V. sinesis*. Shakir *et al*. (2016), also showed that high doses of pesticides above recommended field rate inhibited seed germination in *Lycopersicon esculentum*. Contrarily, Delian *et al*. (2016) observed that treatment of maize seeds with insecticide seed coating agent did not have any effect on their germination rate. However, Defang *et al*. (2010) concluded that the application of modified chitosan had relatively positive impacts on agronomic properties of corn seedlings compared with the conventional toxic seed coating agents.

The effects of coating agents on the average shoot length of *V. unguiculata* seedling are presented in Table 2. Over 5 weeks period of observation, the average shoot length (cm) of experimental cowpea seedlings ranged from 4.82 - 12.85; 3.24 – 12.44; 4.63 – 11.36; 3.25 – 9.70 and 7.55 – 14.83 for the treatment IT, 2IT, BW, 2BW and UT respectively. At the end of 5th week after planting, significant reduction were observed in the shoot length of treated growing seedlings of *V. unguiculata* as compared to control (Table 2).

The average number of leaves of *V. unguiculata* seedlings range from 6 – 14; 3.6 -13.3; 5.7 – 13.0; 2.7 – 12.5 and 6.5 – 14.0 for the treatments IT, 2IT, BW, 2BW and UT respectively (Table 3). The seed coating agents had no significant effect ($p > 0.05$) on the average number of leaves of growing *V. unguiculata* seedling throughout the period of observation. Generally, the significant reduction in the shoot length of all the treated seedling of *V. unguiculata* as compared with control after 5 weeks of planting was an indication of the negative effect of Imidadoprid-Thiram and battery wastes coating agents on the agronomic properties of *V. unguiculata* (Table 2 and 3). Similar evidence had been shown by Aliero *et al*. (2014) who reported that the application of butachlor and pendimethalin pesticides at concentration above 5 mg/ml significantly affect the agronomic parameters of *V. unguiculata*. However, Huang *et al*. (2015) reported that seed coating treatments with neonicotinoid pesticide had no influence on the agronomic properties of oilseed rape seedlings. Likewise, Zaller *et al*. (2016) observed that application of seed treatment agents had no influence on the growth parameter of wheat seedlings. Increases in the yield of *V. unguiculata* were reported by Egho and Enujeke (2012) after the application of insecticide. Recently, a study has shown that the effect of seed coating pesticide on

agronomic properties of plants had a strong relationship with the type of crops under investigation (Milosavljevic *et al*., 2019).

Effect of seed treatment chemicals on rhizobia and phosphate solubilizing bacterial population of the rhizosphere region of Vigna unguiculata

The average counts of rhizobia groups (cfu / g x 10^7) range from 71 – 113; 57 – 102; 79 – 119; 43 – 80; 83 – 115 for the rhizosphere soil samples of the seed treated with IT, 2IT, BW, 2BW and UT respectively (Table 4). At the end of 5th week after planting, significant reduction ($p < 0.05$) in the average numbers of rhizobia groups was observed in the rhizosphere region of the soil treated with IT, 2IT and 2BW (Table 4). While for phosphate solubilizing bacteria, the average counts (cfu / g x 10^7) range from 39 – 66; 31 – 51; 33 – 52; 25 – 46 and 43 – 78 for the rhizosphere soil samples of the seed treated with IT, 2IT, BW, 2BW and UT respectively (Table 5). At the end of 5th week after planting, significant reduction ($p < 0.05$) were observed in the average numbers of PSB groups in the rhizosphere region of all the soil treated with Imidadoprid-Thiram and Battery wastes coating agents (Table 5).

Generally, higher significant negative effect ($p < 0.05$) of seed coating agents on both rhizobia and PSB counts were observed in the rhizosphere region of *V. unguiculata* seedling coated with higher doses of the agents. Studies had shown that treatment of seed with pesticide prior to sowing at the recommended rate did not show any significant effects on soil microbial communities (Ahtiainen *et al*., 2003; Patkowska, 2009). However, the negative impact of seed dressing pesticides on soil microbial biomass was reported by Zaller *et al*. (2016) using the basal respiration method. Janusauskaite *et al*. (2012) reported a significant increase in the bacterial populations of the soil samples collected from the rhizosphere region of the fungicidal treated seed of pea seedling. Ibekwe *et al*. (2001) concluded that the pesticide used for agronomic purpose could have vary impact on soil bacterial community which could range from no effect to long lasting effect and exclusively dependent on the type and concentration of pesticide.

Conclusion

The findings in this research had shown that the application of seed coating agents could have a significant negative effect on some agronomic features of crop plants. Also, the negative effect on the population of beneficial microbial communities could have dire effect on the sustainability of the soil ecosystem for agricultural purposes. Further, research is required to evaluate the impact of these coating agents on the physiology of the cowpea plants.

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Table 1: Germination rate of *Vigna unguiculata* seed as observed on the 3rd, 4th, 5th and 6th days after planting

Treatments (g/kg)	Germination (% / day)			
	3rd	4th	5th	6th
IT	$48^{b} \pm 0.20$	$64^{a} \pm 0.23$	$92^{b} \pm 0.43$	$100^{a} \pm 0.00$
2IT	$22^{c} \pm 0.49$	$44^{b} \pm 0.53$	$72^{d} \pm 0.49$	$80^{b} \pm 0.30$
BW	$52^{b} \pm 0.72$	$72^{a} \pm 0.47$	$88^{c} \pm 0.25$	$98^{a} \pm 0.44$
2BW	$28^{c} \pm 0.23$	$46^{b} \pm 0.26$	$72^{d} \pm 0.28$	$72^{b} \pm 0.23$
UT	$67^{a} \pm 0.42$	$72^{a} \pm 0.27$	$98^{a} \pm 0.43$	$100^{a} \pm 0.00$

Key: IT = Imidadoprid-Thiram; BW = Battery wastes; Values were presented as the $\bar{x} \pm \sigma$ (i.e. Mean plus / minus standard deviation of mean); Mean value with the same letter in the column are not significantly different from one another at 5% level of significant

Table 2: Average shoots length of *Vigna unguiculata* seedling per week after planting

Treatments (g/kg)	Shoot length (cm/week)				
	1st	2nd	3rd	4th	5th
IT	$4.82^{b} \pm 0.13$	$6.99^{b} \pm 0.24$	$9.59^{b} \pm 0.46$	$11.72^{a} \pm 0.18$	$12.85^{b} \pm 0.06$
2IT	$3.24^{c} \pm 0.16$	$5.14^{b} \pm 0.33$	$8.04^{b} \pm 0.53$	$10.74^{b} \pm 0.65$	$12.44^{bc} \pm 0.24$
BW	$4.63^{b} \pm 0.67$	$6.49^{b} \pm 0.45$	$8.02^{b} \pm 0.79$	$9.69^{b} \pm 0.82$	$11.36^{c} \pm 0.30$
2BW	$3.25^{c} \pm 0.71$	$5.10^{b} \pm 0.62$	$6.63^{c} \pm 0.41$	$8.05^{c} \pm 0.30$	$9.70^{d} \pm 0.84$
UT	$7.55^{a} \pm 0.74$	$8.83^{a} \pm 0.21$	$11.15^{a} \pm 0.97$	$12.99^{a} \pm 0.32$	$14.83^{a} \pm 0.97$

Key: IT = Imidadoprid-Thiram; BW = Battery wastes; Values were presented as the $\bar{x} \pm \sigma$ (i.e. Mean plus / minus standard deviation of mean); Mean value with the same letter in the column are not significantly different from one another at 5% level of significant

Table 3: Average number of leave of *Vigna unguiculata* seedling per week after planting

Treatments (g/kg)	Number of leave / week				
	1st	2nd	3rd	4th	5th
IT	$6.0^{a} \pm 0.00$	$8.5^{a} \pm 1.20$	$11.5^{a} \pm 0.25$	$13.3^{a} \pm 0.73$	$14.0^{a} \pm 0.71$
2IT	$3.6^{b} \pm 0.29$	$6.7^{a} \pm 0.67$	$10.0^{a} \pm 0.57$	$12.0^{a} \pm 1.15$	$13.3^{a} \pm 0.27$
BW	$5.7^{a} \pm 0.44$	$8.0^{a} \pm 1.15$	$10.0^{a} \pm 0.54$	$12.6^{a} \pm 0.22$	$13.0^{a} \pm 0.57$
2BW	$2.7^{b} \pm 0.41$	$7.3^{a} \pm 0.42$	$9.3^{a} \pm 0.33$	$10.6^{a} \pm 0.62$	$12.5^{a} \pm 0.34$
UT	$6.5^{a} \pm 0.96$	$9.33^{a} \pm 0.25$	$12.6^{a} \pm 0.37$	$14.0^{a} \pm 0.36$	$14.0^{a} \pm 0.36$

Key: IT = Imidadoprid-Thiram; BW = Battery wastes; Values were presented as the $\bar{x} \pm \sigma$ (i.e. Mean plus / minus standard deviation of mean); Mean value with the same letter in the column are not significantly different from one another at 5% level of significant

Table 4: Effect of seed treatment chemicals on the average counts of rhizobia bacteria in the rhizosphere soils of *Vigna unguiculata* seedlings

Treatments (g/kg)	Average Rhizobial Counts (x 10^{7}) cfu / g				
	1st	2nd	3rd	4th	5th
IT	$71^{a} \pm 0.29$	$90^{b} \pm 0.25$	$108^{b} \pm 0.56$	$113^{b} \pm 0.06$	$110^{b} \pm 0.58$
2IT	$57^{b} \pm 2.17$	$72^{b} \pm 1.32$	$82^{c} \pm 2.96$	$89^{c} \pm 1.48$	$102^{b} \pm 2.14$
BW	$79^{a} \pm 1.32$	$85^{b} \pm 1.41$	$93^{c} \pm 4.51$	$110^{b} \pm 1.63$	$119^{a} \pm 0.37$
2BW	$43^{b} \pm 0.57$	$55^{c} \pm 0.60$	$71^{d} \pm 0.98$	$62^{d} \pm 1.07$	$80^{c} \pm 1.81$
UT	$83^{a} \pm 0.85$	$118^{a} \pm 0.91$	$120^{a} \pm 0.02$	$123^{a} \pm 1.74$	$115^{a} \pm 4.51$

Key: IT = Imidadoprid-Thiram; BW = Battery wastes; Values were presented as the $\bar{x} \pm \sigma$ (i.e. Mean plus / minus standard deviation of mean); Mean value with the same letter in the column are not significantly different from one another at 5% level of significant

Table 5: Effect of seed treatment chemicals on the average counts of phosphatesolubilizing bacteria (PSB) in the rhizosphere region of *Vigna unguiculata* seedlings

Treatments	Average PSB Counts (x 10^7) cfu / g				
(g/kg)	1st	2nd	3rd	4th	5th
IT	$66^b \pm 1.82$	$56^b \pm 6.28$	$45^b \pm 4.08$	$39^a \pm 0.92$	$42^b \pm 3.42$
2IT	$49^c \pm 1.60$	$51^b \pm 7.15$	$43^b \pm 3.92$	$31^b \pm 7.42$	$36^b \pm 4.19$
BW	$52^c \pm 1.66$	$47^b \pm 4.15$	$40^b \pm 3.63$	$33^b \pm 1.08$	$39^b \pm 2.82$
2BW	$46^c \pm 1.37$	$32^c \pm 2.25$	$29^c \pm 2.95$	$25^c \pm 2.68$	$27^c \pm 1.38$
UT	$78^a \pm 1.08$	$65^a \pm 6.95$	$52^a \pm 3.76$	$43^a \pm 2.52$	$49^a \pm 1.88$

Key: IT = Imidadoprid-Thiram; BW = Battery wastes; Values were presented as the $\bar{x} \pm \sigma$ (i.e. Mean plus / minus standard deviation of mean); Mean value with the same letter in the column are not significantly different from one another at 5% level of significant