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Effect of Bacterial Flora on the Viability of Cowpea Seeds Sold in Port Harcourt

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Authors' contributions

This work was carried out in collaboration among all authors. Author ECC designed the study. Author NWEJ managed the analyses of the study performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EOW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Microbial contamination of plants as well as products from plants have become major concern to the public. Contamination of plants could lead to deterioration of the plants including their product thereby altering the nutrient composition of the expected plant product and also presents health hazards if foods from these plants are consumed without much preparation. In Nigeria, cowpea is an important leguminous plant that offers so many forms of delicacies and very rich in protein. The viability and bacterial flora of two cowpea varieties- IT/2246 (brown) and IT/84e (white) purchased from Mile 1, Mile 3, Town market and Rumuokoro market were investigated in the Department of Plant Science and Biotechnology, Rivers State University for proper identification. The cowpea seeds were sorted according to size and wholesomeness. Invitro bacterial assessment of the cowpea seeds was carried out and the bacterial isolates were identified using standard microbiological methods. Viability was done invitro using the cotton wool. Sizeable cotton wool in Petri dishes was dampened in 5ml of distilled water and the different cowpea seeds placed on them. The different sizes and colour of the seeds were placed on the petri dishes. Radicle and plumule lengths were measured for 14days. *Bacillus cereus, Bacillus subtilis* and *Staphylococcus*

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aureus were the bacterial isolates identified. The viability test showed that the radicle length of the big brown, big white, small brown and small white cowpea seeds were 5.12±2.57, 4.75±4.08, 5.66±3.94 and 8.13±3.61 cm for the sample from Mile 1 market. The plumule length of cowpea ranged from 6.91±5.27 to 12.44±5.62, 6.58±2.72 to 9.04±4.9, 4.32±4.29 to 9.15±4.1, and 5.67±5.24 to 10.14±5.4 cm for the Mile 1, Mile 3, Rumuokoro and Town market cow pea samples, respectively. The growth rate of the big brown cowpea, small brown cowpea, big white cowpea and small white cowpea in season one ranged from 40% to 93%, 33% to 73%, 20% to 47% and 40% to 47%, while in season two, 80% to 87%, 73% to 100%, 53% to 73% and 73% to 80% were recorded. The bacterial isolates identified could be pathogenic if food is not properly prepared. Poor pre-harvest seed management practices, poor storage condition and handling processes after harvest could be responsible for the variation in viability.

Keywords: Cowpea; viability; bacterial flora and pathogenicity.

1. INTRODUCTION

Cowpea is a leguminous plant which serves as potential substitute for other protein sources for human consumption due to their high protein content. They are widely cultivated in the humid tropics of South-western Nigeria [1]. The origin of cowpea is not known, but it is believed to have originated from West Africa and South Africa. Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. Some literature indicates that cowpea was introduced from Africa to the Indian subcontinent approximately 2 000 to 3 500 years ago, at the same time as the introduction of sorghum and millet, while others state that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia [2]. Cowpea is widely cultivated in the humid tropics of South-western Nigeria; however, its cultivation is faced with several setbacks such as pests and diseases. The effect of field diseases on cowpea has led to significant reduction in yield of cowpea in the humid forest of Nigeria [3]. The major economic diseases of cowpea in the humid agro ecologies of South-western Nigeria include brown blotch, anthracnose, cercospora leaf spot, choaniphora pod rot, false smut, web blight and sclerotium stem blight [4]. Microbial diseases of cowpea could lead to the breakdown of the cowpea seeds or the crops, or it could cause undesirable change in the property of cowpea due to the vital activities of microorganisms, either directly or indirectly by products of their metabolism [5]. Biodeterioration of cowpea could lead to the loss of nutritional value, organoleptic and color changes, and most importantly, safety may become compromised [5]. The effect of bacterial pathogen on cowpea have been reported to cause the common bacteria blight and fuscous blight. According to Muthii [6], common bacterial blight and fuscous blight of cowpea are serious disease of cowpea in East Africa and the qualitative and quantitative yield losses have been reported to be around 10 to 45%. The disease severity is said to vary depending on the weather conditions and bean cultivar susceptibility. Under fairly high temperatures (25-35°C), high rainfall and humid conditions and the bacteria cause most severe disease [6]. In Ethiopia, it was reported that for every percentage of common bacterial blight severity increase, there was a yield loss of approximately 3.9 to 14.5 kg/ha [7]. Seed-borne bacteria pathogen can survive as long as the seed remain viable. Seed transmission is therefore the primary means by which the pathogen is disseminated [6]. This current study evaluated the effect of the bacterial flora isolated from cowpea seeds on the viability of the seeds.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in four major markets located in two local government areas: Port Harcourt City Local Government Area and Obio-Akpor Local Government Area. These markets are known for high influx of traders who come from different localities to display and sell their produce. The map of the area under study is illustrated in Fig. 1.

2.2 Sample Collection

Cowpea seeds of different variety and sizes (brown and white) were obtained from four major markets in Port Harcourt metropolis from different distributors. The markets were Mile III, Mile I, New market (Borikiri) and Rumuokoro market. The cowpea seeds were taken to the Department of Plant Science and Biotechnology, Rivers State University for proper identification.

In the laboratory, the samples were sorted according to size, and wholesomeness for further analysis [8].

2.3 Isolation and Identification of Bacterial Isolates

The bacterial isolates present in the cowpea seeds were isolated by inoculating aliquot (0.1 ml) of 10^{-1} dilution resulting from 10-fold serial dilution into freshly prepared Nutrient agar plates which were incubated at 37 ℃ in the incubator for 24 hours. Pure bacterial isolates were subcutured, preserved in agar slant at 4˚C and identified using specific biochemical tests [9].

2.4 Pathogenicity Test

Pathogenicity test as described in Koch's postulates was carried out. Bacterial isolates from the cowpea seeds were inoculated onto fresh healthy cowpea seeds. The inoculation was carried out by transferring the bacterium into 20ml sterile distilled water. The turbidity matched the 0.5 McFarland. The water which contained the bacterium was sprayed directly on the healthy cowpea seeds which were kept in sterile Petri dishes. Prior to inoculation of the cowpea seeds, the weight of the healthy cowpea seeds was measured using electronic weighing balance. The weight of the sprayed cowpea seeds was observed by weighing them on an electronic weighing balance (Denver instrument, Germany-090111M) after five days of inoculation to know if the bacterium had an impact on the cowpea seeds. After five days of inoculation of healthy cowpea seeds with bacterial pathogens, the infected cowpea seeds were analysed using microbiological techniques to determine the presence of the inoculated organisms. This was done to ensure that the infections on the cowpea seeds were as a result of the organisms inoculated.

2.5 Germination Test Invitro

Germination test was done invitro using the cotton wool. Sizeable cotton wool was placed on petri dish and 5ml of sterile water was added to allow moisture. The different sizes and colour of the seeds were placed on the petri dishes and the length of radical and plumule were measured for 2, 4, 6, 8 and 10 days.

2.6 Statistical Analysis

Complete Randomized Design (CRD) was used for data analysis. Three treatments: *Bacillus cereus* and cowpea, *B. subtilis* and cowpea and *Staphylococcus aureus* and cowpea were analyzed and ANOVA was used to check for significant difference. Scheffe's Post Hoc test was used for pair-wise comparison between the different seed varieties, and sizes.

3. RESULTS

The result of the pathogenicity test which showed the weight of the different cowpea seeds is presented in Tables 1 and 2. The weight of the cowpea seeds before treatment with *Bacillus cereus* was 0.37g while after treatment the weight reduced to 0.34g. The weight of the control was 0.35g. The weight of the big white cowpea seeds before treatment with *Bacillus cereus* was 0.40g, while after inoculation with *Bacillus cereus,* the weight decreased to 0.37g. The weight of the control was 0.38g (Table 1). The weight of the small brown cowpea seeds before and after inoculation with *Bacillus cereus* were 0.19g and 0.12g, respectively. zIt was observed that the weight reduced from 0.19g to 0.12g, while the weight of the control was 0.18g. It was also observed that the weight of the small white cowpea seeds reduced from 0.19g (before inoculation) to 0.13g (after inoculation) after being treated with *Bacillus cereus,* while the weight of the control was 0.18g (Table 1). In the treatment with *Bacillus cereus,* despite the decreased in the weight recorded, there was no significant difference between the weight of the control, the weight of the cowpea seeds before and after inoculation ($P > .05$).

The weight of the different cowpea seeds before and after treatment with *Bacillus subtilis* showed that the weight of the big brown cowpea seeds before inoculation was 0.38g while after inoculation, the weight reduced to 0.35g as compared to the control which was 0.36g (Table 1). Also, the weight of the small brown cowpea seeds before and after treatment with *Bacillus subtilis* was 0.2g and 0.1g, respectively, while the control was approximately 0.2g. There was significant difference ($P \leq .05$) between the weight of the small brown cowpea seeds before and after treatment with *Bacillus subtilis*. Thus, the weight of the cowpea seeds before treatment with the isolate was higher than the weight after treatment with the isolate (Table 1). Similar observation was drawn from the weight of the small white cowpea seeds which had initial weight of 0.22g reduced to 0.16g (Table 1).

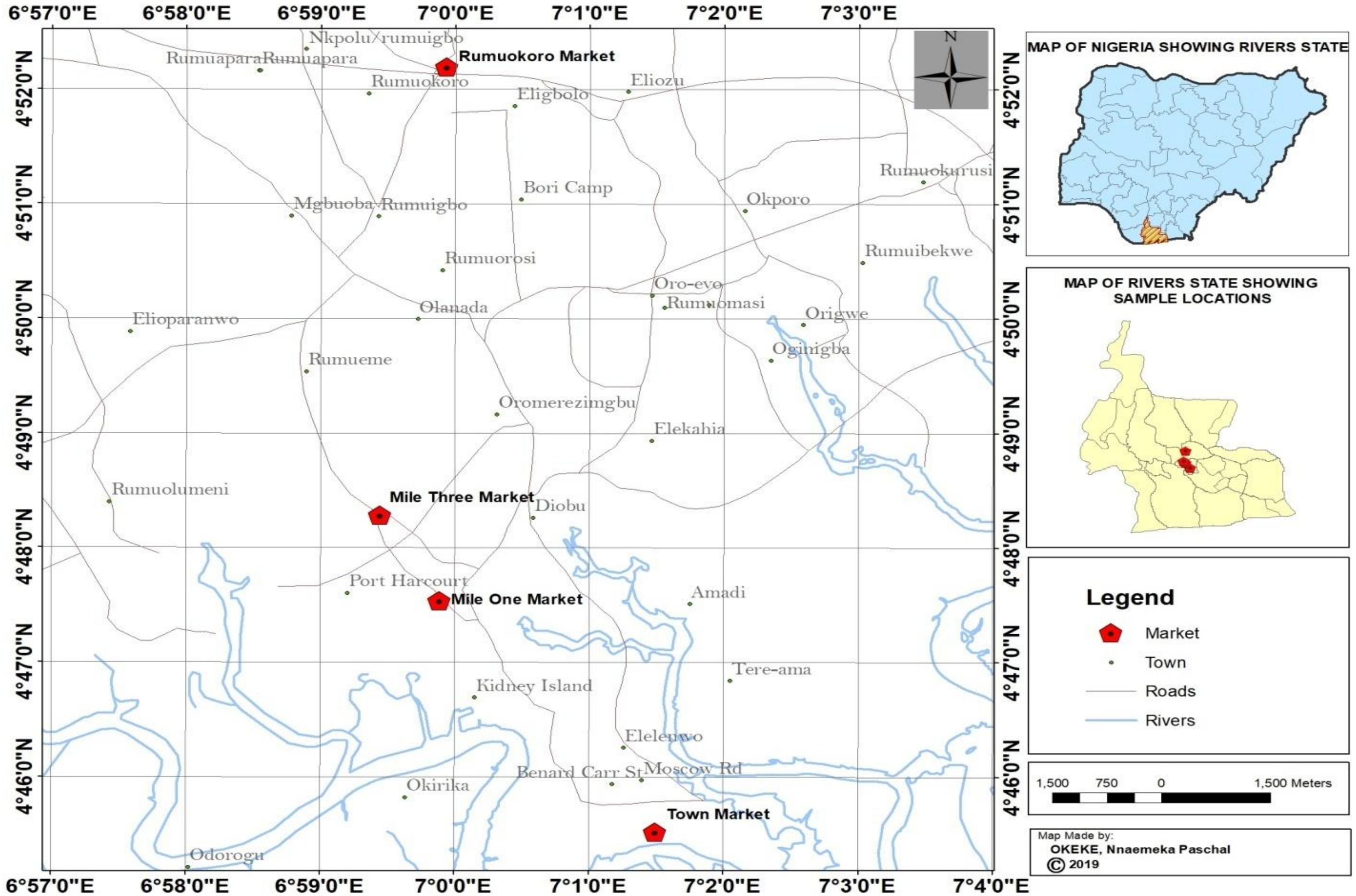

Fig. 1. A map showing the area under study.

Table 1. Pathogenicity test for bacteria on different varieties of cowpea (season 1)

Bacteria	Variety	Weight (g)			
		Control	Before Inoculation	After Inoculation	Weight Loss
Bacillus cereus	BB	0.353±0.08 [a]	0.368±0.08 [a]	0.338±0.06 [a]	0.03±0.01 [a]
	BW	0.38±0.04 [a]	0.395±0.04 [a]	0.368±0.05 [a]	0.027±0.027 [a]
	SB	0.178±0.04 [a]	0.19±0.04 [a]	0.115±0.04 [a]	0.07±0.002 [a]
	SW	0.178±0.06 [a]	0.19±0.06 [a]	0.128±0.05 [a]	0.06±0.01 [a]
Bacillus subtilis	BB	0.358±0.08 [a]	0.375±0.09 [a]	0.35±0.11 [a]	0.03±0.02 [a]
	BW	0.373±0.05 [a]	0.385±0.04 [a]	0.37±0.08 [a]	0.02±0.04 [a]
	SB	0.19±0.032 [a]	0.203±0.03 [a]	0.12±0.02 [a]	0.08±0.01 [a]
	SW	0.208±0.04 [a]	0.223±0.04 [a]	0.155±0.03 [a]	0.06±0.01 [a]
Staphylococcus aureus	BB	0.385±0.04 [a]	0.398±0.03 [a]	0.36±0.05 [a]	0.04±0.03 [a]
	BW	0.378±0.07 [a]	0.39±0.07 [a]	0.345±0.09 [a]	0.04±0.02 [a]
	SB	0.173±0.03 [a]	0.188±0.03 [a]	0.14±0.05 [a]	0.05±0.01 [a]
	SW	0.205±0.02	0.22±0.03	0.16±0.02	0.06±0.00

Means with similar superscript have no significant difference (P ≤.05) Keys: BB: Big brown, BW: Big white SB: Small brown, SW: Small white

The weight of the different cowpea seeds before and after treatment with *Staphylococcus aureus* in season one as illustrated in Table 1, showed that the weight of the big brown cowpea seeds before and after treatment was 0.40g and 0.36g respectively, while the weight of the control was 0.39g. Also, the weight of the big white cowpea seeds before and after treatment was 0.39g and 0.35g, respectively, while the weight of the control was 0.38g. The weight of the small brown cowpea seeds before and after treatment was 0.19g and 0.14g, respectively. The weight of the small white cowpea seeds also reduced from 0.22g to 0.16g, while the control was 0.21g. There was a significant difference between the weight of the cowpea seeds before and after treatment, while there was no significant difference between the weight of the cowpea seeds before inoculation and the weight of the control.

The pathogenicity result showing the weight of the various cowpea (cowpea seeds) before and after treatment with *Bacillus subtilis* and *Staphylococcus aureus* for season two is presented in Table 2. The weight of the big brown cowpea seeds before and after treatment with *Bacillus subtilis* was 0.36g and 0.28g, respectively, while the weight of the control was 0.34g. However, the weight of the big white cowpea seeds reduced from 0.37g to 0.31g, while the weight of the control was 0.36g. Despite the differences observed in the weight loss, there was no significant differences between the respective weight of the cowpea seeds for control, before inoculation and after inoculation. Furthermore, the weight of the small brown cowpea seeds reduced from 0.18g to 0.14g, while the control was 0.17g. Furthermore, the weight of the small white cowpea seeds reduced from 0.24g to 0.14g, while the weight of the control was 0.23g. There was a significant difference between the weight of the cowpea seeds before inoculation and the weight after inoculation, while the weight of the cowpea seeds before inoculation and the weight of the controlled cowpea seeds showed no difference ($P \le .05$).

The weight of the big brown cowpea seeds also reduced from 0.43g to 0.29g after inoculation with *Staphylococcus aureus*, while the control had the weight of 0.42g. There was a significant difference between the weight of the cowpea seeds (before inoculation which was higher) and the weight of the cowpea seeds after inoculation. The weight of the big white cowpea seeds also reduced from 0.43g to 0.33g, while the weight of the control was 0.42g. One-way ANOVA showed that there was significant difference between the weight before inoculation and the weight after inoculation. Similarly, there was a significant difference between the weight before inoculation and the weight after inoculation which saw the weight of the small brown cowpea seeds reduced from 0.20g to 0.14g, while the weight of the control was 0.19g. More so, the weight of the small white cowpea seeds before and after inoculation was 0.20g and 0.18g. Despite the slight loss in weight, there was no significant differences between the weights of the cowpea seeds.

3.1 Percentage Germination

The result showing the percentage germination (rate of the cowpea varieties for season one and season two is presented in Tables 3 and 4, respectively. The growth rate of the big brown cowpea seeds, small brown cowpea seeds, big white cowpea seeds and small white cowpea seeds in season one ranged from 40% to 93%, 33% to 73%, 20% to 47% and 40% to 47%, respectively (Table 3). Also, in season two, the growth rate of the big brown cowpea seeds, small brown cowpea seeds, big white cowpea seeds and small white cowpea seeds in season one ranged from 80% to 87%, 73% to 100%, 53% to 73% and 73% to 80%, respectively (Table 4).

4. DISCUSSION

4.1 Pathogenicity

The bacterial isolates affected the weights of the cowpea varieties thereby causing a loss in weight as compared to the original weight. Though the controls which were not inoculated or treated with any microbial inoculant had slight weight loss but the weight loss were not as those observed with the microbial treated cowpea seeds. The reduced weight observed in this study after treatment with microbial inoculants could be attributed to microorganisms which caused the cowpea seeds to deteriorate. Deterioration of food materials as a result of microorganisms could lead to loss of the physical properties of the food material. This agrees with Hocking et al. [10] who stated that biodeterioration of food materials leads to loss in food physical and chemical properties.

4.2 Percentage Germination

The percentage germination of the cowpea varieties in season two were higher than the values observed in season one except the big brown cowpea seeds which had higher germination percentage in samples from Rumuokoro market for season one. Factors which could have caused the variations in germination rate could be related to poor pre-harvest seed management practices, poor storage condition and handling processes after harvest. This agrees with reports by previous

Table 2. Pathogenicity test for bacteria on different varieties of cowpea (season 2)

Bacteria	Varieties	Weight (g)			
		Control	Before Inoculation	After Inoculation	Weight Loss
Bacillus subtilis	BB	0.34±0.043[a]	0.355±0.042[a]	0.283±0.051[a]	0.08±0.011[a]
	BW	0.355±0.097[a]	0.368±0.095[a]	0.313±0.061[a]	0.05±0.04[a]
	SB	0.17±0.047[a]	0.18±0.047[a]	0.143±0.026[a]	0.04±0.02[a]
	SW	0.225±0.037[a]	0.238±0.033[a]	0.143±0.021[a]	0.10±0.01[a]
Staphylococcus aureus	BB	0.418±0.033[a]	0.428±0.033[a]	0.295±0.025[a]	0.13±0.008[a]
	BW	0.418±0.04[a]	0.433±0.045[a]	0.333±0.057[a]	0.1±0.012[a]
	SB	0.19±0.022[a]	0.2±0.022[a]	0.135±0.024[a]	0.06±0.002[a]
	SW	0.188±0.034[a]	0.2±0.035[a]	0.183±0.017[a]	0.02±0.018[a]

Means with similar superscript have no significant difference (P ≤.05). Keys: BB: Big brown, BW: Big white, SB: Small brown, SW: Small white

Table 3. Percentage germination of cowpea varieties from different markets for season I

Markets	BB (%)	SB (%)	BW (%)	SW (%)
Mile I	40	73	20	47
Mile II	87	60	47	40
Town	60	33	27	47
Rumuokoro	93	73	33	40

Keys: BB: Big brown, BW: Big white, SB: Small brown,SW: Small whit

Table 4. Percentage germination of cowpea varieties from different markets in season II

Markets	BB (%)	SB (%)	BW (%)	SW (%)
Mile I	87	100	73	73
Mile II	87	73	60	80
Town	87	100	53	80
Rumuokoro	80	80	53	73

Keys: BB: Big brown, BW: Big white, SB: Small brown, SW: Small whit study that poor storage conditions from preharvest and post-harvest practices could affect the germination percentage of cowpea [11]. Also, the moisture content of the cowpea could be a factor that affected the cowpea seeds. This agreed with studies conducted in Ethiopia by [12] who reported that seeds stored in containers (tin pots) maintained the moisture content of the seeds which gave high percentage germination than those stored in jute bags or polythene bags. Furthermore, the germination percentage of the small brown cowpea seeds was higher than the values reported by [11] which ranged from 60.6% to 93.5%. Also, the values reported in the same study varied slightly with those reported in this current study. The germination tests of the length of the plumule and the radicles of the seeds varied respectively amongst the different cowpea varieties and the markets. In season one, the radicle length of the cowpea seeds in Mile 1, Mile 3 and Town market varied slightly (Tables 5 and 6) but there was no significant difference with the different radicle lengths of the cowpea, whereas the statistical analysis showed that the radicle length in the Rumuokoro market had slight significant differences (Table 5). For instance, the radicle length of the big white cowpea was the least and was significantly different (*P ≤.05)* from the radicle lengths of the big brown, small white and small brown cowpea. Similar to the observations drawn from the radicle lengths, the plumule lengths of the different cowpea varied amongst the cowpea varieties and across the locations for season one (Tables 7 and 8). Despite the slight variations in the plumule length of the cowpea varieties in the Mile 3 and Town markets, there was no significant difference across the cowpea (*P ≤.05*). Whereas the plumule lengths of the Mile 1 and Rumuokoro markets significantly varied. For instance, in the Mile 1 market, the plumule length of the small white cowpea which was the highest length was significantly different from the plumule length of big white cowpea (Table 6). More so, the plumule length of the big white which had the least length was significantly different from the plumule length of the big brown cowpea (Table 6). The plumule length of the different cowpea obtained in this study were lower than the 22.5cm and 23.0cm reported by Kedir et al. [11] who assessed the quality of common cowpea seed quality in Ethiopia. Also, the radicle length in this current study are lower than the 17.0 cm and 17.5cm reported by previous study [11]. Furthermore, work done by previous authors agreed with this current study that the radicle length, the plumule length of the cowpea seeds varied amongst the seed varieties [11,13]. The variations in the lengths of the radicle and plumule in this current study could be attributed to the quality of the seeds as well as the moisture content [13].

Table 5. The Radicle Length (cm) of the cowpea in season one

Varieties	Mile 1	Mile 3	Rumuokoro	Town
BB	$5.12±2.57^a$	$5.12±2.39^a$	$4.53±1.8^a$	$5.99±4.03^a$
BW	$4.75±4.08^a$	$5.09±2.12^a$	$2.25±2.1^b$	$5.15±3.37^a$
SB	$5.66±3.94^a$	$4.93±2.26^a$	$4.37±1.71^a$	$6.47±4.07^a$
SW	$8.13±3.61^a$	$3.85±2.07^a$	$4.23±1.47^a$	$3.37±3.52^a$

Means with similar superscript have no significant difference (P ≤.05), Keys: BB: Big brown, BW: Big white SB: Small brown, SW: Small white

Table 6. Plumule length(cm) of the crops in season one

Varieties	Mile 1	Mile 3	Rumuokoro	Town
BB	$9.75±4.32^{ab}$	$9.04±4.9^a$	$9.15±4.1^a$	$9.07±5.4^a$
BW	$6.91±5.27^b$	$6.58±2.72^a$	$4.32±4.29^b$	$8.43±4.5^a$
SB	$9.37±4.98^{ab}$	$7.29±2.29^a$	$6.69±5.1^{ab}$	$10.14±5.4^a$
SW	$12.44±5.62^a$	$7.52±3.55^a$	$8.19±4.36^{ab}$	$5.67±5.24^a$

Means with similar superscript have no significant difference (P ≤.05), Keys: BB: Big brown, BW: Big white SB: Small brown, SW: Small white

Table 7. Radicle Length(cm) of the crops in season one

Sample Locations	BB	BW	SB	SW
Mile 1	5.12±2.57^{a}	4.75±4.08^{a}	5.66±3.94^{a}	8.13±3.61^{a}
Mile 3	5.12±2.39^{a}	5.09±2.12^{a}	4.93±2.26^{a}	3.85±2.07^{b}
Rumuokoro	4.53±1.8^{a}	2.25±2.1^{b}	4.37±1.71^{a}	4.23±1.47^{b}
Town	5.99±4.03^{a}	5.15±3.37^{a}	6.47±4.07^{a}	3.37±3.52^{b}

Means with similar superscript have no significant difference (P ≤.05)

Table 8. Plumule Length (cm)of the crops in season one

Sample Locations	BB	BW	SB	SW
Mile 1	9.75±4.32^{a}	6.91±5.27^{a}	9.37±4.98^{a}	12.44±5.62^{a}
Mile 3	9.04±4.9^{a}	6.58±2.72^{a}	7.29±2.29^{a}	7.52±3.55^{b}
Rumuokoro	9.15±4.1^{a}	4.32±4.29^{a}	6.69±5.1^{a}	8.19±4.36^{ab}
Town	9.07±5.4^{a}	8.43±4.5^{a}	10.14±5.4^{a}	5.67±5.24^{b}

Means with similar superscript have no significant difference (P ≤.05), Keys: BB: Big brown, BW: Big white SB: Small brown, SW: Small white

5. CONCLUSION

The negative effect of *Bacillus subtilis, Bacillus cereus,* and *Staphylococcus aureus,* on the quality of cowpea have been demonstrated. Thus, contamination of these isolates on cowpea seeds could lead to loss in quality and could also affect the rate of germination. Furthermore, most of the isolates in this study could be pathogenic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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