

**SEED INFECTION ROUTS OF COWPEA BACTERIAL BLIGHT  
INDUCED BY *Xanthomonas axonopodis* pv. *vignicola* (BURKHOLDER)  
DYE.**

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**SUMMARY**

The significant role played by seed-borne infection calls for more studies on seed infection routes of cowpea bacterial blight, since epidemics have been reported on newly opened areas; suggesting seed transmission rather than infected plant debris or soil contamination. Although it is understood that cowpea bacterial blight is seed transmitted, and that seed-borne inoculum play a significant role in the disease epidemiology, the mechanism of transmission from seed to emerging seedling has not been fully elucidated. The objective of the studies was to study seed infection routes of cowpea bacterial blight (*Xanthomonas axonopodis* pv. *vignicola*). Direct systemic infection of seed by the pathogen was investigated using artificial inoculation and spray-inoculated at vegetative state (25 days after sowing) method while indirect systemic infection was determined by at spray-inoculated at flowering, flat pod and mature pod stages with bacteria suspension adjusted to *ca.*  $4.7 \times 10^7$  cfu ml<sup>-1</sup> while one seed lot soaked in/spray with sterile distilled water was used as control. Harvested pods were sorted into symptom categories using scale 1-9 and threshed accordingly. Hundred seeds each of the harvested seeds were plated on YDCA media and planted in the screen house to examine the presence of *Xav* and percentage infected seedlings were calculated. While the population of *Xav* in/on was determined by seed destruction assays. The study indicated that the bacterium was able to transmit systemically from inoculated

**seeds to harvested seeds and epiphytically from inoculated vegetative parts into the harvested seeds.**

**Keywords:** Admixture, seed, induced, infection, bacterial, blight, roots

**COWPEA**, *Vigna unguiculata* (L.) Walp, is an annual herbaceous legume belonging to the family Fabaceae order Leguminosae and genus *Vigna* (26). Cowpea is grown in tropical and subtropical regions of the world, covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, Southern USA, and Central and Southern America, lying between 35 °N and 30 °S of the Equator (10). It is an important food legume, and an essential component of cropping systems in the tropics. The dominant crop mixtures include millet- cowpea, sorghum-cowpea, maize- cowpea and millet-cowpea - groundnut. Being a fast-growing crop, cowpea curbs erosion by covering the ground, fixes atmospheric nitrogen, and its residues contribute to soil fertility. An important agricultural advantage is that cowpea is able to fix nitrogen in the soil efficiently at about 30-70 kg/ha. Nutritionally, cowpea has excellent food value that includes carbohydrate (55-60 %), protein (23-27 %), fiber (3.9 %), ash (2.77-3.6 %) and fat (1.3 %), depending on genotypes and environmental factors (23). The digestibility of cowpea protein was found to be 85 %, compared with 95 % biological value

of egg albumen. The per capita consumption is 25 to 30 kg per annum per Nigerian (10). Part of the popularity of cowpea as a source of protein for many people is the low cholesterol; a major problem associated with consumption of animal protein. Most developed countries with high rates of atherosclerosis, cancer, diabetes, hypertension, and obesity individuals are advised to change diets and consume more vegetables (including legumes) than meat.

Cowpea bacterial blight, also known as bacterial canker, remains one of the most damaging diseases that significantly reduce yield of cowpea wherever the crop is grown (13). Up to 60 % seedling mortality has been reported in cowpea due to infected seeds (13). Some losses may be attributed to pod lesions, which can expand to the seed, if infection occurs during pod development and can result in rotting and shriveling of the seed (30). Severity of yield loss varies according to cultivar, levels of infecting environment and stage of growth (24). *Xanthomonas axonopodis* pv *vignicola* (Xav) is a warm climate pathogen, favoured by a temperature range of 28-32 °C and

high relative 0-80 % (11). Infection occurs naturally through stomata, hydathodes and wounds; resulting in the disease symptoms (9).

Seeds are regarded as a highly effective means for transporting plant pathogens over a long distance. Most of these seed - borne pathogens could have severe economic and ecological consequences if they are introduced and become established in regions where they are not native. The pathogen may thus, be carried with the seeds as admixture, externally on seed surfaces and internally when pathogens are established within the seeds with definite relationship. The seed - borne pathogen may result in loss of germination, discoloration and shriveling, development of plant diseases and toxin production in infected seeds (2).

The seed consists of the embryo, storage tissue/cotyledons and seed coat. The establishment of a pathogen in any part of the seed described above is referred to as seed infection. The process of seed infection is influenced by the conditions under which the crop grows (8). Seed - borne micro-organisms can be considered in four ways. The first, consists of pathogen for which the seed is the main source of inoculum, the second class consists of important pathogens in which the seed - borne phase of the disease is of minor

significance as a source of inoculum, the third and largest group of seed - borne micro-organisms consists of those that have never shown to cause disease as a result of their presence on seeds, the fourth class is a group of micro-organisms that can infect the seed either in the field or in storage and reduce yield and seed quality (7, 8).

The significant role played by seed-borne infection calls for more studies on seed transmission of Cowpea bacterial blight (CoBB), since epidemics have been reported on newly opened areas, suggesting seed transmission rather than infected plant debris or soil contamination (8). Although it is understood that CoBB is seed transmitted, and that seed-borne inoculum play a significant role in the disease epidemiology, the mechanism of transmission from seed to emerging seedling has not been fully elucidated. A major step toward effective management of this disease would require an understanding of seed infection routes of cowpea bacterial blight from seed to the emerging seedling. Deciphering the seed infection routes of cowpea bacterial blight from the seed to the emerging seedling provides the necessary basis to set up new management approach. The objective of the studies was to study seed infection routes of bacterial blight

induced by *Xanthomonas axonopodis* pv. *vignicola*.

## **MATERIALS AND METHODS**

### **Experiment 1: Direct infection of seeds by the pathogen through the vascular system**

Direct systemic infection of seed by the pathogen was investigated using artificial inoculation method. Two cowpea varieties, Ife brown and SAMPEA 7 were used. The seeds were divided into 3 seed lots of 40 seeds each of the varieties. One seed lot was soaked in bacteria suspension adjusted to *ca.*  $4.5 \times 10^7$  cfu /ml<sup>-1</sup> before planting. Other seed lots were spray-inoculated at vegetative state (25 days after sowing) with bacteria suspension adjusted to *ca.*  $4.7 \times 10^7$  cfu ml<sup>-1</sup> while one seed lot soaked in sterile distilled water was used as control. Five seeds per pot were planted in 25 cm diameter pot filled with sterilized soil. These were thinned to two plants per pot after seedling establishment. Harvested pods were sorted into symptom categories using scale 1-9 (22), and threshed accordingly. Hundred seeds each of the harvested seeds were plated on YDCA media and planted in the screen house to examine the presence of *Xav* and percentage infected seedlings were calculated. While the population of *Xav* in/on was determined by seed destruction assays (17).

### **Experiment 2: Indirect systemic infection via floral parts**

In order to determine the indirect systemic infection in the floral parts, twelve pots each of the two cowpea varieties were raised in the screen house. Bacterial suspensions adjusted to *ca.*  $4.5 \times 10^7$  cfu/ml were prepared. The suspensions were sprayed by hand held atomizer sprayer at flowering stage. After spray inoculated 2 mm<sup>2</sup> of floral parts (flower stalk, calyx, receptacle, corolla, carpel and ovary) were harvested at random and were macerated and serially diluted and plated on YDCA after 24, 48 and 72 h of inoculation. At maturity pods were harvested. The harvested pods were grouped according to disease severity rating scale 1-9 (22). Percentage aborted flower and pod were calculated accordingly as described previously. Hundred seeds each from symptom groups were plated on YDCA media and planted in the screen house to examine the presence of *Xav* and to calculate the rate of transmission and percentage infected seedlings. The population of *Xav* was determined by seed destruction assays (17).

### **Experiment 3: Indirect seed infection via pod inoculation at flat pod and matured pod stages**

Five seeds per pot each of cowpea varieties (Ife brown and SAMPEA 7) were planted in a 25 cm diameter pot filled with sterilized soil. These were

watered and fertilized as required. Cowpea plants were spray inoculated at flat pod and when the pod was ripped for harvest, with bacterial suspension adjusted to  $ca.4.7 \times 10^7$  cfu ml<sup>-1</sup>. The control seedlings were sprayed with sterile distilled water. Polyethylene bags were used to cover areas that were not inoculated and all inoculated parts were tagged accordingly. The experiments were laid out in CRD with 10 replications. The experiments were repeated once. In order to determine the distribution of *Xav* in material tissues that leads to internal and external seed colonization, fertilized young pod and matured pods were randomly collected a 2 mm<sup>2</sup> section of fruit coat, fruit stalk, dorsal suture, funicle, and testa were macerated and were serially diluted and plated on YDCA for colony enumeration. The relevance of the work was to demonstrate the colonization pattern of the immature and mature pod during the various stages of seed development. Harvested pods and seeds were processed as described in section previously. The experiment was conducted in the screen house of the Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria. Data collected were analyzed statistically using analysis of variance (ANOVA) and means were separated by means of LSD.

## **RESULTS**

Seed inoculation produce more symptomatic pods than vegetative inoculation. In Table 1 however, there was statistical difference between all the treatments in terms of germination in both symptomatic and symptomless pods.

Table 2 shows the infection of CoBB after flower inoculation. After 24 h of inoculation *Xav* population vary from  $0.5 \times 10^5$  in stalk to  $2.3 \times 10^8$  in corolla in Ife brown while in SAMPEA 7, the population varies from  $0.8 \times 10^5$  in stalk to  $1.3 \times 10^8$  in corolla which was the highest population. In Table (3), germination ranges from 50 % in symptomatic pod to 58 % in symptomless pod in Ife brown but was statistically similar to SAMPEA 7 (47-55 %). Similar result was also obtained in seedling establishment. The percentage infection was higher in symptomatic pods (75.00 % and 85.00 %) respectively in Ife brown and SAMPEA 7 while in symptomless pods the rate of transmission was 65.00 % in Ife brown and 55.00 % in SAMPEA 7. There was pod abortion in flat pod inoculation and there was no pod abortion in mature pod inoculation. The flat pod inoculation leads to high pod infection in both varieties SAMPEA-7 and Ife brown (Table 5). There were no obvious or conspicuous symptoms on pods following mature pod inoculation.

The epiphytic population of *Xav* was higher in mature pod inoculation than the flat pod inoculation. The percentage of infection was higher in flat pod inoculation (75 % and 80 %) compared to (10 %) in matured pod inoculation. Similar result was obtained in population of *Xav*. Unshed flower led to canker development on pod. After flower

inoculation canker symptom was observed on pods, and pod suture. Pod shattering symptoms were observed after flat pod inoculation. Heavy seed infection from symptomatic pods resulted to early CoBB symptoms on the emerging cowpea seedling.

**Table 1:** Transmission of *Xanthomonas axonopodis* by symptomatic and symptomless pod

Variety	Treatment	Symptomatic pods				Symptomless pods			
		Germination (%)	Seedling establishment (%)	Infection (%)	Population (%)	Germination (%)	Seedling establishment (%)	Infection (%)	Population (%)
Ife brown	Seed inoculation	67.00b	35.50e	50.00b	2.3x10 <sup>2</sup> a	65.00c	57.00d	20.00b	2.0x10 <sup>3</sup> a
	Vegetative inoculation	65.00c	57.00b	30.00d	1.4x10 <sup>2</sup> b	80.00b	60.10c	11.50d	0.7x10 <sup>3</sup> c
SAMPE A 7	Seed inoculation	65.00c	37.50d	52.00a	2.5x10 <sup>2</sup> b	64.60d	55.60e	22.00a	1.2x10 <sup>3</sup> b
	Vegetative inoculation	63.00d	47.00c	32.00c	1.3x10 <sup>2</sup> b	80.00b	62.40b	13.00c	0.5x10 <sup>3</sup> d
Control		100a	100a	0.00e	0.00c	100a	100a	0.00d	0.00e
SE		1.91	3.00	0.42	1.91	3.50	3.20	0.52	0.40

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

**Table 2:** Population of *Xanthomonas axonopodis* on the floral parts after 24, 48, and 72 hours after inoculation

Treatment/ after Variety inoculation	Hours	Floral parts					
		Stalk	Calyx	Receptacle	Corolla	Carpel	Ovary
Ife brown	24	0.5x10 <sup>5</sup>	1.2 x 10 <sup>5</sup>	1.4 x 10 <sup>6</sup>	2.3 x	0.00	0.00
	48	1.5x10 <sup>6</sup>	1.6 x 10 <sup>8</sup>	2.5 x 10 <sup>9</sup>	10 <sup>5</sup>	1.0x10 <sup>6</sup>	1.3x10 <sup>6</sup>
	72	1.6x10 <sup>6</sup>	1.7 x 10 <sup>8</sup>	2.7 x 10 <sup>9</sup>	3.0 x 10 <sup>9</sup>	1.0x10 <sup>6</sup>	1.5x10 <sup>6</sup>
SAMPEA 7	24	0.8	1.5 x 10 <sup>5</sup>	1.3 x 10 <sup>6</sup>	1.3 x	0.00	0.00
	48	x10 <sup>5</sup>	1.4 x 10 <sup>8</sup>	2.7 x 10 <sup>9</sup>	10 <sup>8</sup>	1.2x10 <sup>6</sup>	1.2x10 <sup>6</sup>
	72	1.4 x	1.6 x 10 <sup>8</sup>	3.0 x 10 <sup>9</sup>	2.9 x 10 <sup>9</sup>	1.0x10 <sup>6</sup>	1.4x10 <sup>6</sup>
Untreated SAMPEA 7		10 <sup>6</sup>			3.3 x 10 <sup>9</sup>		
		0.00	0.00	0.00	0.00	0.00	0.00

Means in a column followed by the same letter are not significantly different at 5 % level of significance using LSD test

**Table 3:** Transmission of *Xanthomonas axonopodis* to seed from symptomless and symptomatic pods following inoculation at flowering stage combined analysis of two trials

Treatment	Symptomless pod				Germination	Symptomatic pod		
	Seedling establishment	Rate	Population	Population		Seedling establishment	Rate	Population
Ife brown	58b	46b	65a	3.6x10 <sup>3</sup> b	50b	29c	0.75b	2.7x10 <sup>7</sup> a
SAMP EA 7	55c	35c	0.55	3.7x10 <sup>3</sup> a	47c	35b	0.85a	2.6x10 <sup>7</sup> b
Control	100a	100a	0.00	0.00c	100a	100a	0.00c	0.00c
S. E	1.50	2.32	0.25	1.23	3.2	2.44	0.21	1.23

Means in a column followed by the same letter are not significantly different at 5 % level of significance using LSD test.

**Table 4:** Population of *Xanthomonas axonopodis* (cfu/g) on parts of cowpea pods at different times of inoculation

Variety	Treatment	Hours after inoculation	Floral parts						
			Stalk	Fruit coat	Dorsal suture	Funicle	Testa	Cotyledon	
Ife brown	Flat pod inoculation	24	2.4x10 <sup>8</sup> b	2.7x10 <sup>8</sup> a	2.8x10 <sup>8</sup> a	0.00	0.00	0.00	
		48	1.2x10 <sup>8</sup> a	2.8x10 <sup>8</sup> a	3.4x10 <sup>9</sup> a	2.2x10 <sup>9</sup> a	0.00	0.00	
		72	1.3x10 <sup>8</sup> b	2.9x10 <sup>8</sup> a	3.4x10 <sup>9</sup> a	2.3x10 <sup>9</sup> a	0.00	0.00	
		96	1.3x10 <sup>5</sup> a	2.9x10 <sup>6</sup> a	3.5x10 <sup>7</sup> a	2.4x10 <sup>6</sup> a	0.00	0.00	
	Matured pod inoculation	24	1.0x10 <sup>8</sup> d	1.1x10 <sup>8</sup> c	2.2x10 <sup>8</sup> c	0.00	0.00	0.00	
		48	1.1x10 <sup>8</sup> b	2.6x10 <sup>8</sup> b	2.4x10 <sup>9</sup> c	2.0x10 <sup>9</sup> ac	0.00	0.00	
		72	1.4x10 <sup>8</sup> a	1.5x10 <sup>8</sup> c	1.3x10 <sup>9</sup> d	1.0x10 <sup>8</sup> d	0.00	0.00	
		96	1.1x10 <sup>3</sup> b	1.2x10 <sup>5</sup> c	1.5x10 <sup>5</sup> c	1.4x10 <sup>5</sup> c	0.00	0.00	
	SAMPEA-7	Flat pod inoculation	24	2.6x10 <sup>8</sup> a	2.6x10 <sup>8</sup> b	2.8x10 <sup>8</sup> a	0.00	0.00	0.00
			48	1.0x10 <sup>8</sup> c	2.5x10 <sup>8</sup> c	3.0x10 <sup>9</sup> b	2.1x10 <sup>9</sup> b	0.00	0.00
			72	1.3x10 <sup>8</sup> b	2.8x10 <sup>8</sup> b	3.2x10 <sup>9</sup> b	2.2x10 <sup>9</sup> b	0.00	0.00
			96	1.3x10 <sup>5</sup> a	2.7x10 <sup>6</sup> b	3.4x10 <sup>6</sup> b	2.3x10 <sup>6</sup> b	0.00	0.00
Matured pod inoculation		24	1.1x10 <sup>8</sup> c	1.0x10 <sup>8</sup> d	2.4x10 <sup>8</sup> b	0.00	0.00	0.00	
		48	1.0x10 <sup>8</sup> c	2.4x10 <sup>8</sup> d	2.4x10 <sup>9</sup> c	2.1x10 <sup>9</sup> b	0.00	0.00	
		72	1.2x10 <sup>8</sup> c	1.3x10 <sup>8</sup> d	1.4x10 <sup>9</sup> c	1.2x10 <sup>9</sup> c	0.00	0.00	
		96	1.0x10 <sup>3</sup> c	1.3x10 <sup>5</sup> d	1.4x10 <sup>5</sup> d	1.2x10 <sup>5</sup> d	0.00	0.00	
Control		0.00e	0.00e	0.00e	0.00e				
S. E. ±		1.20	1.30	1.32	1.25				

Means in a column followed by the same letter are not significantly different at 5 % level of significance using LSD test

**Table 5:** Seeds infection and population growth following inoculation at flat and matured pod, two trials combine analysis

Treatment		% infection	Population(cfu)
Flat pod inoculation	Ife brown	75	2.6x10 <sup>7</sup> a
	SAMPEA 7	80a	2.5x10 <sup>7</sup> b
Mature pod inoculation	Ife brown	10c	0.2x10 <sup>2</sup> c
	SAMPEA 7	10c	0.3x10 <sup>2</sup> d
	Control	0.00d	0.00e
S. E		3.45	0.32

Means in a column followed by the same letter are not significantly different at 5 % level of significance using SNK test.

## DISCUSSION

The higher percentage of non-infection in the vegetative stage of inoculation was due to the inability of the bacteria to move into the systematic parts of the plant and spread systemically in the vascular system (27). This was due mainly to the fact that movement of bacteria into internal parts of the plant were influenced by many factors such as wetness of the leaves caused by rain, duration of wetness, and injury caused by insects or other animals (6). In the screen house however, these conditions were not readily available. The higher percentage of pod infection in seed inoculation was due mainly to the fact that bacteria pathogen can invade the vascular tissue of the host and spread

systemically in the xylem, reaching the pods and seeds through the funiculus (12). The percentage infection 50 % in symptomatic pods compared to 0.20 in symptomless pods (Table 2) was the cause of low seedling establishment (35.50 and 37.50 %) and high population of *Xav* observed. High pod infection can lead to high seed infection (30). Heavily infected seed can lead to high seedling manifesting symptoms (plate 16). Though the percentage infection of *Xav* (20 %) and population (10<sup>3</sup>) was low in symptomless pods, the significant reduction of seedling establishment (55.60 %) compared to 100% in the control treatments was an indication that bacteria can cause serious epidemics even at low population

level (21). The general lower rate of transmission and population of *Xav* observed in vegetative inoculation was due to the age of the host plants. These results corroborate the report of Singh and Munoz (25) that stages of crop maturity at infection affect the spread of the disease.

The population of *Xav* within the floral parts (Table 2) indicated that flower receptacle and corolla had the highest population ( $10^6$ ). This could be due to the elevated nutrients in these parts of the plants. The results agree with the report of Ngugi and Schem (18) that flowers are considered excellent microbial habitats, being well supplied with nutrients. The bacteria were not detected in carpel and ovary because they had not yet colonized the floral parts down to this area being the inner parts of flower. At 48 h after inoculation all the floral parts were infected and the population ranges from ( $10^6$ ) in flower stalk, carpel and embryo sac to ( $10^8$ ) in calyx and ( $10^9$ ) in receptacle and corolla. Similar results were also obtained 72 h after inoculation. There was high percentage of flower aborted at 7 and 14 DAI. This development might be due to the accumulation of the pathogen in the vessels thereby clogging and prevent free flow of water and nutrients to the flower (30). Similarly, Darsonval *et al.* (7) reported that the ability of bacteria to

form biofilm in planta leads to the congestion of some rare vessels that culminated to leaf shedding, flower and fruit abortion and the general wilting of plants. The high rate of transmission (75 % and 85 %), high population ( $10^7$  and low seedling establishment 29 and 35 %) was due to high seed infection resulting from symptomatic pods (Table 3). This result corroborate the finding of Cafati and Seattler (5) who reported that in symptomatic pods, the blight organisms may enter the pod cavity via the stomata of the pod or by breaking through the vascular tissues of the pod suture; the bacteria then pass into the funiculus and the raphe or the microphyle leading into the seed. Plate 1, 2 and 3 shows the canker symptoms on stems and pods. Canker develop when bacterial pathogen invades the xylem vessels, impairing water transport and lead to tissues death (29). Though very low ( $10^3$ ) population of bacteria titre was obtained in the symptomless pods, indicating that bacteria transmission did not entirely depends on the pod symptoms. This result agrees with the earlier work of Mabagala (15) that CoBB has the ability to enter pods through vascular system and infect the seed without causing lesions on the surface of the pods.

The low germination percentage 58 and 55 % compared to the control 100 % low seedling establishment 46 and

35 % compared to the control 100 % and high rate of transmission 65 % and 55 % in symptomless pod indicated that bacteria pathogenicity on seeds had little to do with population but more to do with virulence strain of the bacteria, and the location of bacteria in/on the seeds (20). The significant difference ( $P \leq 0.05$ ) between the flat pod inoculation and mature pod inoculation indicated the susceptibility of the pod area to *Xav* at different stages of development (27). The absence of *Xav* in the testa and cotyledons was due mainly to the fact that bacteria pathogen lack the ability to directly penetrate through the testa and the cotyledons unlike the fungal pathogens (19). However, the absence of bacteria in testa and embryo might suggest that there is only a limited 'window' in the developmental process during which bacteria can enter. Such a 'window' might easily differ with the physiological status of the host plant and could account in part for the observed differences in these results (16). The high population of *Xav* in dorsal suture and funicle was due to these sites acts as an umbilical cord to the seeds making the place rich in nutrients. This finding suggests that seed infection was primarily a sequel of pod colonization.

The lower population of *Xav* observed in mature pod inoculation

might be due to the pod being lignified at this stage (19). This was in line with the report of Singh and Munoz (25) who reported that severity of yield loss varies according to levels of infection, environment and stage of crop growth and maturity at infection. There was no varietal resistance observed in this result. Aggour *et al.* (1) reported that cowpea genotypes may show resistance in leaves, but susceptible in pods, or vice versa. Bacteria tend to move and spread faster through vascular of succulent tissues of susceptible hosts (25). These results corroborate the report of Goodwin (12) that infection of pod at early state of pod development can result in pod abortion, rotting, shattering and shriveling of the seed (plate 4, 5 and 6). Pod abortion will be the end product of bacterial pathogen in vascular system of pod because the bacteria will block the movement of water and nutrients to and within the pods by depositing extracellular polysaccharide (EPS), secretion of toxin and the formation of biofilm in the vessels (7). The gradual reduction in pod abortion 14 DAI and general absence of pod abortion in mature pod inoculation may be due to corresponding lignification of pod tissues which hinder the free movement of the pathogen (19).

Flat pod inoculation leads high seed infection while there was no infection in mature pod inoculation. Severity of

pod infection varies with stage of pod maturity at infection (25). The high population of *Xav* ( $10^4$ ) in symptomatic pod might be due to biofilm formation on the pods by *Xav* pathogen. This result parallel the report of Barak *et al.* (4) that population of bacteria recover from the surface of the plant tissue was higher than the population recovered from the inside of the plant tissue. Aggregation followed by biofilm formation is a strategy used by *Xav* during colonization of bean phyllosphere for its protection from stresses and maintenance of inoculation reservoirs (14). If *Xav* is present, the epidemic of bacterial blight disease should be driven almost completely by the suitability of conditions for multiplication of the pathogen in association with leaves or pods in the field (27). The bacteria penetrate the pods via pods stomata or wounds and remain in the apoplast (intercellular spaces) of infected tissues or remain deposited on the pods. The epiphytic populations serve as reservoirs and contribute to either secondary or primary infection (28). The higher transmission rate and *Xav* population in flat pod inoculation may be due to the succulent nature of pod tissue at that stage and also the stomata openings are large enough for active penetration and passive entrance of bacterial cells (6). Conversely, the low rate and low population of *Xav* in mature pod

might be attributed to mechanical barriers to infection as a result of lignification. The toughness of epidermis was due to the polymer of cellulose, hemicelluloses, lignin, and polymerized organic compounds (6). Bacteria can adhere to the pod surface and multiply epiphytically through bacteria surface constituent such as lipopolysaccharides which are the principal components of the outer membrane of gram-negative bacteria (3). This result is in line with the report of Mabagala (15) that foliage and pod of beans are known to harbor relatively high population of plant pathogenic bacteria. These phyllosphere bacteria can lead to seed contamination and infection. The pathogen itself can stick or get mixed with seeds during any of the processes during seed harvesting, extraction, threshing and packaging (19).

## **CONCLUSION**

The study of route of seed infection showed that seed inoculation, vegetative inoculation, flower inoculation, flat pod inoculation and mature pod inoculation gave rise to infected pods and seeds, indicating the capability of *Xav* to move from plant surface to the internal tissue and systemically transmit to seeds. Flower and flat pod inoculation gave rise to heavy flower and pod abortion. Severely infected pods produce high infected seeds but pod lesions is not

an index to measure the level of seed infection since symptomless pods also gave rise to infected seeds. Seeds are the passive carriers of a diversified microbial cohort in commensal or parasitic relationship.

Deciphering the routes of seed infection and transmission mechanisms of plant-pathogenic bacteria to hosts provides the necessary basis to set up new management approach.

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