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Full Length Research Paper

Assessment of the stability and genotype-environment interaction of a spider plant (*Cleome gynandra* L.) collection in Burkina Faso: Application of the AMMI and GGE models

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Spider plant, *Cleome gynandra* L (Cleomaceae) is an important traditional leafy vegetable in Burkina Faso diets. Due to its high nutritional value and medicinal properties, it is a good dietary supplement for combating nutritional deficiencies and certain degenerative diseases. However, the lack of quality seeds and poor agronomic performance are limitations to crop improvement. The aim of the present study was to evaluate the agro-morphological performance of a collection of *C. gynandra* in relation to the three climatic zones of Burkina Faso. Thus, 36 accessions collected in the three climatic zones of Burkina Faso were evaluated using a Fisher block design. The trials were conducted in August 2019 during the rainy season in the country's three climatic zones. Measurements and observations were made on the traits of interest, including fresh biomass (BMF). The best agronomic performances were recorded at the Bobo Dioulasso Experimental Station, followed by Ouagadougou, while the poorest performances were noted at Dori. The stability test (AMMI and GGE biplot) enabled us to identify accessions adapted to each climate and six high-performing, stable accessions (OUA9, OUA10, KOU, KOM2, BOB3 and MAN) for all three climatic zones. These high-performing, stable accessions can then be popularized among local populations.

Key words: *Cleome gynandra,* agronomic performance, yield in fresh biomass, AMMI, Burkina Faso.

INTRODUCTION

The nutritional composition of *Cleome gynandra* L. (Cleomaceae) makes this crop an added value to be taken into account in achieving food and nutritional security for consumers (Meda et al., 2013; Chand et al.,

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2022). *C. gynandra* is also of economic importance to growers and traders, generating substantial income for market gardeners (Sakande et al., 2022).

Spider plant (*C. gynandra* L.) is of great pharmacological interest and could be used in the treatment of numerous diseases. Its leaves are said to facilitate childbirth and lactation (Mishra et al., 2011). Indeed, in many communities, to facilitate childbirth, a decoction or infusion of the boiled leaves and/or roots is administered to women during labor (Berhaut, 1976; Mishra et al., 2011). This same decoction taken after childbirth is said to promote milk secretion and compensate for blood lost during childbirth thanks to its rich iron content (Bosire, 2014). Iron and vitamin A richness are particularly important health issues in countries plagued by anemia, mainly caused by malaria (Kahane et al., 2005; Moyo and Aremu, 2022). Similarly, according to Mnzava and Chigumira (2004), an infusion of the leaves consumed as a drink treats anemia.

A better understanding of the diversity of *C. gynandra* is therefore essential for the design of initiatives to preserve and promote this crop (Marcia et al., 2014). Thus, agro-morphological characterizations of *C. gynandra* collections have been carried out but on a single site located in the Sudano-Sahelian zone (Kiébré, 2016). These agro-morphological characterizations made it possible to identify the plant's socio-cultural services for local populations. As a result, the effect of genotype-environment interaction on trait expression has not been assessed. Given that phenotype expression is the result of the combined action of genotype, environment and genotype-environment interaction, the behavior of an accession may vary, significantly, from one environment to another (Wasonga et al., 2015). It is, therefore, important that trials be carried out in Burkina Faso's three climatic zones in order to offer farmers *C. gynandra* varieties that are adapted to them. This work is part of the drive to identify accessions adapted to each climatic zone. It therefore aims to evaluate the agromorphological performance of *C. gynandra* collection in relation to the three climatic zones. Specifically, the aims are (i) to determine the effect of accession-experimental site interaction on the variability of *C. gynandra* traits of interest; (ii) to identify high-performance accessions for each of the three climatic zones; and (iii) to identify a batch of high-performance accessions for all three climatic zones.

MATERIALS AND METHODS

Plant

The plant material consists of 36 *C. gynandra* accessions collected in 2019 from gardeners in the three agro-climatic zones of Burkina Faso, namely the Sudanian, Soudano-Sahelian and Sahelian zones. As *C. gynandra* is still in protoculture, the number of accessions varies from one zone to another. Thus, six accessions were collected in the Sudanian zone, three accessions in the Sahelian zone and 27 accessions in the Sudano-Sahelian zone (Table 1).

Experimental sites

Trials were carried out at three sites in the three climatic zones of Burkina Faso.

Site 1

It is located in the Sudanian zone in the peri-urban area of Bobo Dioulasso at 11°12'0" North latitude and 4°18'0" West longitude. The average rainfall recorded during the 2019 trial in Bobo Dioulasso was around 1371 mm of water. Wooded savannah dominates the region, after gallery forest and grassland.

Site 2

It is located in the Soudano-Sahelian zone at Gampela, 18 km east of Ouagadougou on the Ouaga-Niamey axis at 12°15' North latitude and 1°12' West longitude. The average rainfall recorded during the 2019 trial, in Ouagadougou, was 852.7 mm of water. The vegetation is characterized by wooded and grassy savannah.

Site 3

It is located in the Sahelian zone at Djomga 8 km South of Dori on the Dori-Gorom-Gorom axis at 14°02′07″ latitude and 0°02′04″ longitude West North. The average rainfall recorded during the 2019 trial, in Dori, was 509.7 mm of water. The vegetation is characterized by tree and shrub steppe.

The soil types of Ouagadougou (Gampèla) and Bobo Dioulasso (Lafiabougou) are ferric lixisols (sandy-loam texture) according to the WRB (2006) classification. These soils have a strong weathering of the surface horizons and clay accumulation in a deep horizon called argillic horizon (WRB, 2006). The soils of Dori (Djomga) are aerosols with a high sand content.

Experimental design and cultivation practices

On all three sites, an incomplete Fisher block design was used. Each replicate was subdivided into two sub-blocks of 18 accessions. Repeats and successive sub-blocks were separated by 1 m. Within each block, each accession was represented by a 3 m line on which seven seed pots were sown at a rate of 10 seeds/ poquet. Accessions were randomly assigned to lines. The row spacing, and the spacing between bunches were 0.5 m, respectively.

Each plot was ploughed, harrowed, and levelled before planting. An organic amendment, at a rate of 6 tonnes per hectare, was applied to enable the plants to better express their potential (Kiébré et al., 2019). Manual sowing was carried out on August 09, 2019, for the Bobo site, and on August 12, 2019, for the Ouagadougou and Dori sites. A total of three weeding operations were carried out at each site. Demariage was carried out on the 10th day after sowing, with one plant/poquet.

Data collection

Quantitative variables were measured at 45 days after sowing. We determined the *C. gynandra* trait of interest, namely fresh biomass. Fresh biomass (BMF) was determined by weighing each plant in the field immediately after harvesting tender leaves and twigs.

Table 1. Accessions classification used, their morphotypes, and climatic origin.

No.	Genotype code	Morphotypes	Climate zones
1	OUA9	Green	Sudan-Sahel
2	OUA10	Green	Sudan-Sahel
3	OUA1	Green	Sudan-Sahel
4	OUA3	Green	Sudan-Sahel
5	OUA2	Green	Sudan-Sahel
6	BOB3	Green	Sudanese
7	KOU	Green	Sudan-Sahel
8	KOM1	Green	Sudan-Sahel
9	KOM2	Dark purple	Sudan-Sahel
10	OUA6	Green	Sudan-Sahel
11	GAN	Green	Sudan-Sahel
12	BOB2	Green	Sudanese
13	REO2	Light violet	Sudan-Sahel
14	MAN	Light violet	Sudan-Sahel
15	TEN	Light violet	Sudan-Sahel
16	DED2	Dark purple	Sudan-Sahel
17	ZOU	Light violet	Sudan-Sahel
18	DED3	Dark purple	Sudan-Sahel
19	OUA7	Green	Sudan-Sahel
20	GOU	Light violet	Sudan-Sahel
21	BOB4	Light violet	Sudanese
22	DED1	Dark purple	Sudan-Sahel
23	KAY2	Green	Sahelian
24	OUA5	Green	Sudan-Sahel
25	OHG	Dark purple	Sahelian
26	BOB1	Dark purple	Sudanese
27	DED4	Dark purple	Sudan-Sahel
28	FAD	Light violet	Sudan-Sahel
29	MOG	Green	Sudan-Sahel
30	BOND	Green	Sudanese
31	ZOR	Green	Sudan-Sahel
32	KOM3	Dark purple	Sudan-Sahel
33	KAY1	Light violet	Sahelian
34	REO 1	Light violet	Sudan-Sahel
35	DED5	Dark purple	Sudan-Sahel
36	BOB6	Light violet	Sudanese

Data analysis

Gen12ed software was used to perform metanalysis with the mean of fresh biomass yields to determine stability coefficients, propose AMMI and GGE biplot models.

Thus, the stability coefficient (Pi) is an estimate of the genotype's adaptability over a range of environments. According to Hannachi et al. (2019), it is calculated using the high-yielding genotype in each environment as the reference point. Genotypes with the greatest difference in yield from the reference genotype would have the highest Pi value (Lin and Binns, 1988). The most interesting genotypes would be those with the lowest Pi values, most of which would be attributed to genetic deviation (Lin and Binns, 1988).

Environments are ranked by measuring stability, which is given by the superiority of the genotype compared with the mean for each environment. This method is based on the estimation of IPCA2, which measures the probability that the performance of a given genotype is superior to the others (Vasconcelos et al., 2010). It also classifies environments according to IPCA2 as either favorable or unfavorable.

The AMMI model comprises an additive part (the mean, the effect of genotype, and the effect of environment), and a non-additive, multiplicative part (the G×E interaction). The AMMI method combines analysis of variance and principal component analysis (PCA). This model was developed by Zobel et al. (1988). First, the main effects of genotypes and environments (the additive part of the model) are estimated by an analysis of variance. Next, a PCA is performed on the non-additive part of the model, that is, the G×E interaction. As for the GGE biplot model, according to Yan et al. (2000), it essentially models the genotype effect associated with the

Table 2. Stability coefficient of 36 *Cleome gynandra* accessions evaluated.

Accessions	Pi	Accessions	Pi	Accessions	Pi
OUA10	3	OUA2	68	ZOR	89.8
OUA9	5.2	DED1	74.4	BOB6	90.6
MAN	17	OUA6	75.2	OUA5	92.8
KOU	17	BOB1	78.1	FAD	95.2
BOB3	18	REO2	79.1	KOM3	97.4
KOM2	19.1	OUA1	82.2	MOG	98.3
REO1	51.9	BOB2	84.1	KOM1	98.6
BOB4	55.9	OUA7	85.2	GAN	99.3
BOND	59.7	DED3	85.3	OHG	99.8
DED4	60.8	DED5	85.6	GOU	99.9
DED2	63.4	ZOU	85.6	KAY2	102.1
TEN	64.2	OUA3	88.2	KAY1	112.4

Pi: Stability coefficient.

G×E interaction. It was used to identify mega-environments, high-performing and stable genotypes. Statistical significance tests for the genotypic, environment and genotype × environment interaction components were calculated using Fisher's F-test.

RESULTS

Adaptability of accessions and classification of the three environments

The stability coefficients (Pi) of the 36 accessions are shown in Table 2. Accessions OUA9, OUA10, KOU, MAN, BOB3 and KOM2 had the lowest stability coefficients (Pi). On the other hand, accessions GAN, OHG, GOU, KAY2 and KAY1 with high stability coefficients are less stable.

The IPCA2 values (Table 3) show that the environments of Ouagadougou and Bobo-Dioulasso with positive IPCA2 values are favorable environments for growing *C. gynanda*. On the other hand, the Dori environment, with a negative IPCA2 value, is an unfavorable environment.

Effects of the combined analysis of variance of 36 accessions evaluated in three environments

The results of the combined analysis of variance of the 36 accessions evaluated in the three climatic zones according to the AMMI model show significant differences in yield linked to the effects of genotype, environment and genotype-environment interaction ($p < 0.001$). Of the total variation, 45.53% was explained by the environment, 30.45% by the genotype effect and 13.94% by the genotype × environment interaction. Most of the variation in the genotype × environment interaction is explained by the first two components IPCA1 (60.06%) and IPCA2 (39.74%) (Table 4). For the accession-environment interaction, the F-test is highly significant ($p < 0.001$) for the first axis IPCA1 and for the second axis IPCA2 ($p < 0.001$).

The AMMI biplot gave a model fit of 88.28% (Figure 1). Thus, the environments of the Bobo-Dioulasso and Ouagadougou sites contrast with the environment of the Dori site, and by the size of their positive scores. These environments are the most interactive. Accessions OUA2, DED2, DED4, BOB4, OUA9, OUA10, MAN, KOU BOB3 and KOM2 showed high yields and positive IPCA2 scores in the Bobo Dioulasso and Ouagadougou environments. In contrast, accessions KAY1, KAY2, GAN, GOU and OHG showed a negative IPCA2 score, with below-average yield and an IPCA1 score close to zero.

Distribution of accession effect and G×E interaction effect of 36 *C. gynandra* accessions in mega environments

The GGE biplot is presented with two principal components explaining a total of 88.28% of the GGE variation (PC1 69.97%, PC2 18.31%) (Figure 2). The first principal component is represented on the x-axis, and opposite its value is the estimated yield, that is, accessions with higher PC1 values are considered more productive. The second principal component is represented on the y-axis and shows the stability of the accessions. Thus, the Dori and Ouagadougou environments are located on the plane: they constitute a mega-environment. The Bobo-Dioulasso environment, located within the circle, is a mega-environment in its own right. The mega-environment is the most discriminating for the accessions, as indicated by the longest distance between their positions and the point of origin.

Accessions OUA3 and OUA2, positioned at the top of

Table 3. Environment classification.

Environment	Average leaf biomass yield	IPCA2	Class
Bobo Dioulasso	68.32	2.85	Favorable
Dori	26.43	-7.08	Unfavorable
Ouagadougou	72.99	4.23	Favorable

ICPA2= Second principal component of the interaction.

Table 4. Combined analysis of variance in fresh biomass yield of 36 *Cleome gynandra* accessions evaluated in the three environments.

Source of variation	ddl	SC	CM	F_test	Variation explained	G×E
Treatments	107	280429	2621	23.29**		
Accessions	35	94959	2713	24.11**	30.45	
Environments	2	141994	70997	54.43**	45.53	
Blocks	6	7827	1304	11.59**		
Interactions	70	43476	621	5.52**	13.94	
IPCA1	36	26113	725	6.45**		60.06
IPCA2	34	17363	511	4.54**		39.94
Residual	0	0				
Error	210	23634	113			
Total	323	311889	966			

Ddl: Degree of freedom; SC, sum of squares; CM, mean square; ICPA1= first principal component of the interaction and ICPA2= second principal component of the interaction; **highly significant. G×E: genotype-environment interaction.

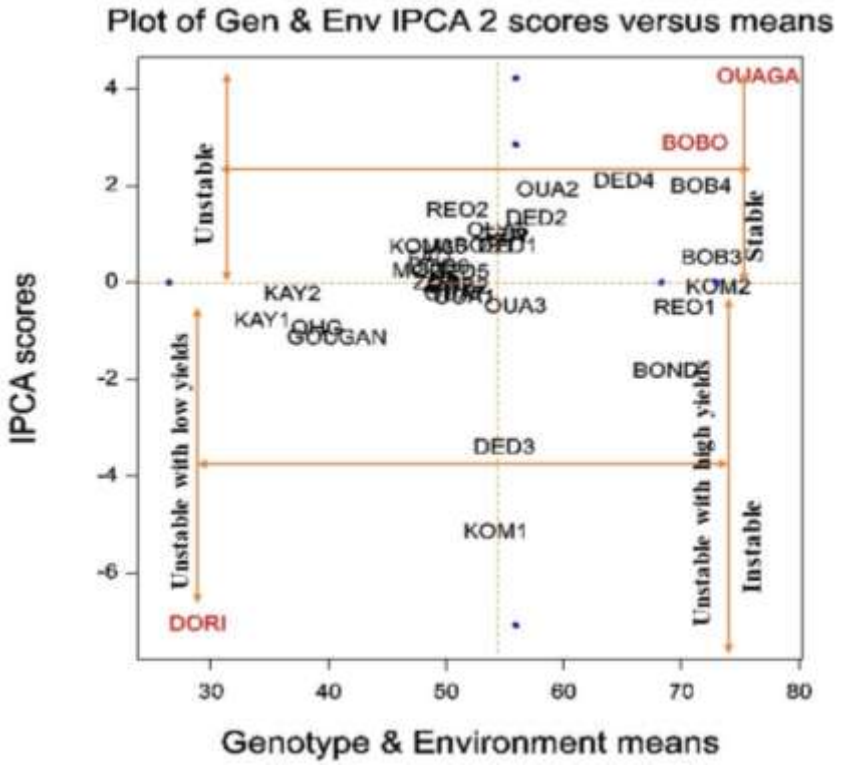

Figure 1. Distribution of the 36 accessions according to the three environments and their IPCA2 genotypic and environmental scores.

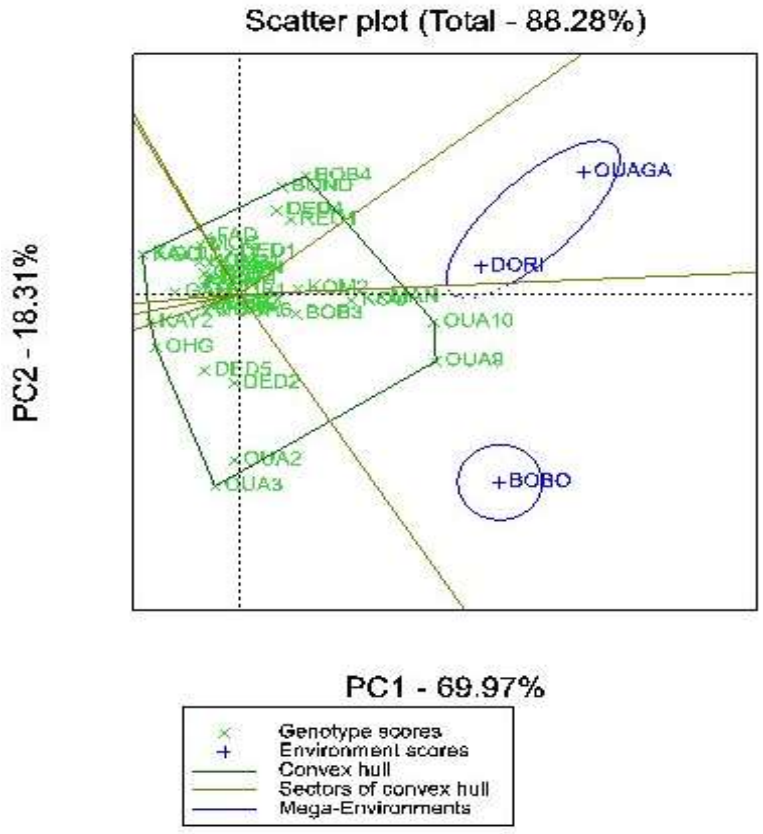

Figure 2. Polygonal view of the GGE (Genotype and genotype-environment interaction) biplot showing the effect of genotype and the effect of GxE interactions of 36 *Cleome gynandra* accessions in three environments.

the polygon and close to the Bobo Dioulasso environment, perform well in this environment. On the other hand, accessions BOB4, BOND, OUA9 and OUA10 positioned at the top of the polygon and in the mega-environment (Ouagadougou-Dori) perform well in this mega-environment. Accessions KOM2, BOB3, KOU, MAN, OUA9 and OUA10 are close to both mega-environments. On the other hand, accessions KAY2, OHG, GOU, GAN and KAY1 are different from the other genotypes in the biplot, due to their positions away from the center and on the left-hand side. They are also unstable and have low yields.

Identification of "ideal accessions" for all three sites

The results of the biplot comparison of accessions in Figure 3 showed that accessions KOU, MAN, BOB3, KOM2 and OUA1, followed by OUA9 and OUA10, which are very close to the AEC center of the concentric circles. They showed the best performance in terms of fresh biomass yield. The other accessions, namely KAY2, OHG, GOU and GAN, are far from the AEC center of the GGE biplot circle. In fact, these accessions performed poorly agronomically, making them undesirable.

DISCUSSION

The AMMI and GGE biplot models revealed significant effects for environment, genotypes and genotype-environment interaction based on leaf biomass yield. This confirms phenotypic diversity among the accessions evaluated, and that these accessions respond differently to environments. The first principal component (IPCA1) of the AMMI model, explained 60.06% of the sum of squared deviations of the interaction. The residual of the model is not significantly different from the weighted error. These results indicated that genotype and site scores on the first principal component of the interaction (IPCA1) explain more than half of the interaction present in the matrix of leaf biomass yield data submitted for analysis. The AMMI biplot of IPCA1, and average genotype and site effects show how each genotype shapes its yield (additive effect only or additive+ multiplicative), and which locality is the best suited.

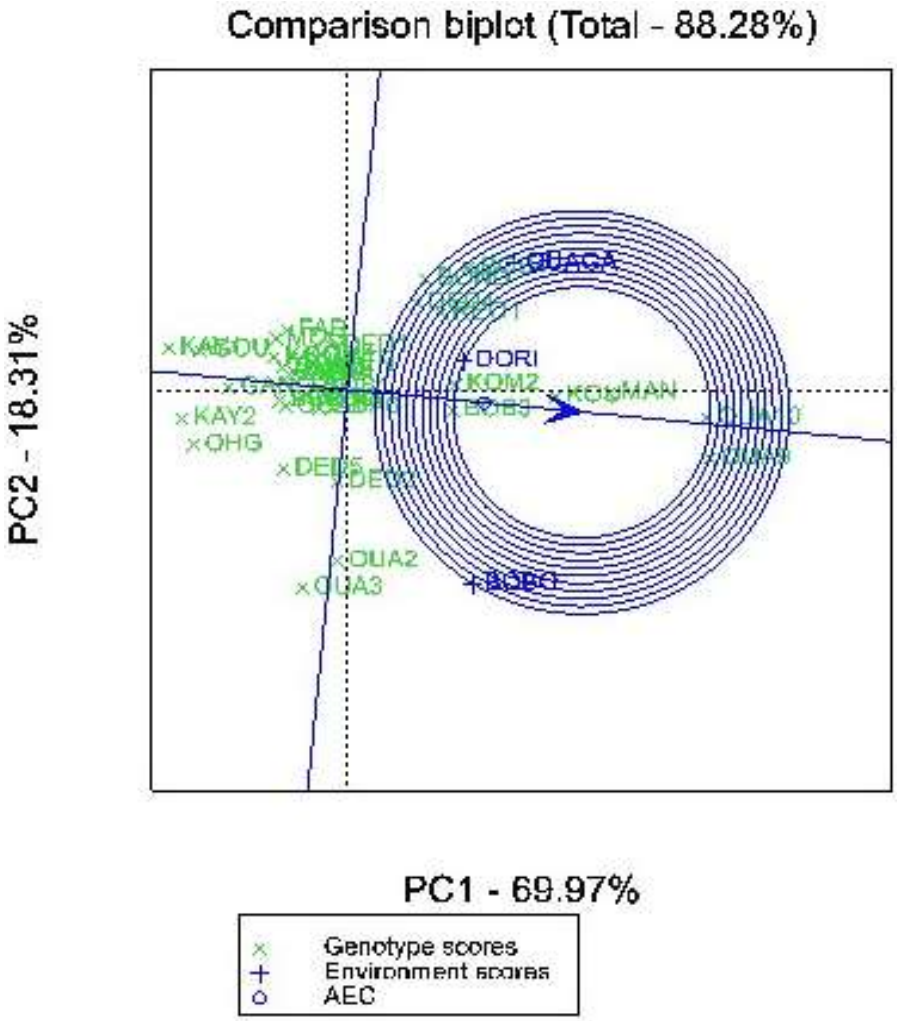

Figure 3. Comparison biplot showing the two main axes of interaction (IPCA2 vs IPCA1) of 36 accessions evaluated in three climatic zones of Burkina Faso for "ideal accessions".

According to Zobel et al. (1988), genotypes with high positive or negative scores show strong interactions. They are specifically adapted to the environment with the score of the same sign. Thus, the AMMI analysis indicated that accessions OUA2, DED4, BOB4 and DED2 were productive; while OUA9, OUA10, BOB3, KOU, MAN and KOM2 were stable in the environments tested. Furthermore, these accessions, with their lower superiority indices and positions close to the origin of the biplot, show a general adaptation to all localities. According to Lin and Binns (1988), the most interesting genotypes would be those with the lowest Pi values, most of which would be attributed to genetic distance. Furthermore, Mohammadi and Amri (2008), in a study of genotype × environment interaction in durum wheat, revealed that genotypes far from the biplot center have a high G × E interaction, while those closer to the biplot center are more stable.

The AMMI graph shows that Ouagadougou and Bobo Dioulasso, with their high scores, better discriminate between the performances of the different accessions evaluated and are a significant source of contribution to the interaction. The Dori site, on the other hand, contributes significantly less to the interaction. The GGE biplot model showed that accessions with PC1 values close to zero show greater adaptability, and genotypes with higher PC1 values are better suited to sites with PC1 values of the same sign. Thus, accessions BOB4, BOND, DED4 and DED1 are more productive in the Ouagadougou and Dori environments; while accessions OUA3 and OUA2 are productive in the Bobo Dioulasso environment. According to Mitrovic et al. (2012), performance assessment of individual genotypes can be based on their position relative to the X (high yield of accessions) and Y (stability of accessions) axes. In this case, the best accessions (OUA9, OUA10, MAN, KOU,

KOM2 and BOB3) are considered those with high yield and stable performance, in all three climatic zones. Thus, they could be used in a research program for high-yielding varieties for extension in Burkina Faso's three climatic zones. Indeed, one of the major factors in the adoption of interesting varieties is superior agronomic performance (Vom Brocke et al., 2010). High yields of fresh biomass are an added value for food and nutritional security, and for market gardeners' incomes. Two mega-environments have emerged, the Bobo-Dioulasso mega-environment and the Ouagadougou-Dori mega-environment. According to Kendall et al. (2019), for studies conducted in different environments, if there is no difference between two or more environments, they are in the same circle and referred to as a mega-environment. It is then recommended to work in one of these environmental groups in subsequent studies. In this case, for future studies on *C. gynandra,* the Dori and the Ouagadougou sites constitute a single environment.

Conclusion

The AMMI biplot and GGE biplot offered three possible alternatives for the breeder. The first is to adopt stable, high-performance accessions such as OUA10, OUA9, MAN, KOM2, KOU, and BOB3. The second alternative is to use the interaction positively, through the choice of accessions. The third alternative is to assign a specific genotype to each site. Under this scenario, we select accessions OUA9, OUA10, BOB4, MAN, DED4, KOU, REO1, BOND, KOM2, and BOB3 for the Ouagadougou site, and accessions OUA9, OUA10, OUA2, OUA3, MAN, KOU, DED2, BOB3, KOM2 and DED5 for the Bobo Dioulasso site, and accessions OUA10, MAN, KOU, KOM1, OUA9, BOND, DED3, REO1, KOM2 and BOB3 for the Dori site. The best accessions identified for each climatic zone and for all three climatic zones could be used to develop high-performance of *C*. *gynandra* varieties in Burkina Faso.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Efficacy of organic extracts of *Nicotiana tabacum* (Solanaceae) leaf and *Jatropha curcas* (Euphorbiaceae) seed against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) under laboratory conditions

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The maize weevil is a pest of stored maize controlled with synthetic insecticides. The widespread use of these insecticides, however, is harmful to the environment and human health. In this study, solvent extracts (SEs) and powder treatments (PTs) of *Nicotiana tabacum* leaves and *Jatropha curcas* seeds were tested for their efficacy against *Sitophilus zeamais* Motschulsky. The experiment was designed in a completely randomized design with three replications. For extraction, ethyl acetate and dichloromethane were used. Extract concentrations of 25, 50, 75 and 100% were evaluated at dosages of 4, 8, and 12 ml. PT of botanicals was evaluated at dosages of 4, 8, and 12 g. Mortality was calculated at 1, 3, 5, 7, and 14 days after treatment. At the end of the experiment, the percentage of grain damage, weight loss, and germination percentage was calculated. Dichloromethane SEs of *N. tabacum* leaves and *J. curcas* seeds at 50, 75, and 100% levels of extract concentrations (LEC) in all doses caused 100% mortality. The *J. curcas* seed PTs at 12 g and its SEs of ethyl acetate and dichloromethane in 100% LEC at 12 ml experienced the least grain weight loss of 1.36, 0.79 and 0.64%, respectively. The results suggest that these plant-based products are very promising, generally available, cost-effective, non-toxic to non-target organisms, and simple to produce. Thus, dichloromethane SEs of *N. tabacum* leaves and *J. curcas* seeds at 50% LEC and above in all doses can be recommended for the management of *S. zeamais*.

Key words: Adult emergence, dichloromethane, ethyl acetate, insecticidal activity, maize weevil.

INTRODUCTION

Maize (*Zea mays* L.) is a member of the grass family (Poaceae). It is one of the world's most significant annual cereal crops, serving as staple food and a source of income for many people in developing countries (Tandzi

and Mutengwa, 2019). Maize grain is damaged by insects, reducing its benefits (Hiruy and Getu, 2020). *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is one of the most damaging insects to stored grain, particularly in tropical areas (Bhusal and Khanal, 2019).

To protect their maize from insect pests, farmers and maize traders use a variety of pest control methods. Synthetic insecticides are the most commonly used grain protection option (Njoroge et al., 2014). On the other hand, insecticide exposure has been linked to several human acute and chronic diseases (Kandel et al., 2021). Hence, it is critical to develop more effective alternatives that control pest populations while minimizing the negative impact of synthetic insecticides (Goldel et al., 2020). Plants and plant-derived products have long been used to control pests in underdeveloped countries (Grzywacz et al., 2014) because they are a more environmentally friendly and cost-effective pest control method than synthetic insecticides (George et al., 2014; Tavares et al., 2021). They are non-toxic to non-target organisms and have a specific mode of action. Furthermore, farmers can easily develop these (Iqbal et al., 2021).

Tobacco, *Nicotiana tabacum* (Solanaceae), is an herbaceous annual or perennial plant in the Solanaceae family (Rao et al., 2016). It has been known for a long time that tobacco leaves are used as insecticides and pesticides (Weber et al., 2019; Kanmani et al., 2021; Prommaban et al., 2022). *Jatropha curcas* belongs to the family Euphorbiaceae (Abdelgadir and Staden, 2013). It is rich in phytochemicals and studied for toxic effects (Oseni and Akindahunsi, 2011). Its extract has a wide range of biological effects, including molluscicidal, acaricidal, and insecticidal effects (Cordova-Albores et al., 2016). Organic extracts of *J. curcas* seeds are shown to be toxic to a wide range of insect orders (Lepidoptera, Coleoptera, Diptera, and Hemiptera) and can be used to protect field crops and stored grains (Temitope, 2014; Eisa et al., 2020). The current study aimed to determine the efficacy of organic extracts of *N. tabacum* leaves and *J. curcas* seeds on maize weevil (*S. zeamais s*) mortality, adult emergence, and grain loss.

MATERIALS AND METHODS

Experimental site and laboratory condition

The study was carried out between July and November, 2022 in the crop protection laboratory of the Bako National Maize Research Centre (BNMRC). Bako is located at 9°6' North and 37°9' East at an elevation of 1650 m above sea level. The experiments were carried out in a laboratory at 25 to 28°C, 65 to 70% relative humidity, and a12:12 (light:dark) photoperiod. The artificial climate chamber controlled the indoor air temperature and relative humidity with errors of ±0.3°C and ±2% throughout the experiment. The experiment was designed in a completely randomized design (CRD) with three replications. As indicated in Table 2, there were 24 treatments (2 botanicals × 4 LECs × 3 doses) for solvent extracts (SEs) and 6 treatments (2 botanicals × 3 doses) for powder treatments (PTs), as indicated in Table 7.

Plant samples collection

Tobacco, *N. tabacum* leaves, and *J. curcas* seeds were collected from gardens near Bako town and dried in shade for 3 to 4 weeks. It was ground into a fine powder using a mortar and pestle. To prevent quality loss, the powder was sealed in polythene bags and kept in the refrigerator (Khan et al., 2014). The botanicals were chosen because they were easily accessible in the area, and their powder tests showed a promising result for storage and insect pest control (Prommaban et al., 2022).

Preparation of dry extracts

To prepare the dry extracts, the solvents ethyl acetate (ETOAc) and dichloromethane (DCM) were used to extract the powdered leaves and/or seeds. For ETOAc extraction, 500 g of powdered leaves and/or seeds were put separately in conical flasks. 1000 mL of ETOAc were added to each flask and corked. The mixture was left for 12 h. After 12 h, the extract filtrates were filtered into separate, labeled bottles using a funnel and Whatman filter paper No. 1. Again 500 mL of solvent was added to each flask (decanted extract), which was then kept for 24 h. After 24 h, the filtration process was repeated. Once more, 500 mL of ETOAc was added to each decanted extract and left for 48 h; the final extract filtrates were collected. Spinning was sometimes done to ensure extraction was complete. To keep solvent from escaping, cotton wool, and aluminium foil were always used to cover the flasks. Dry extracts were created after vacuum-concentrating extract filtrates with a Heidolph rotary evaporator. Dry extracts were formed by drying the concentrates further and removing any remaining solvents (Khan et al., 2014; Gitahi et al., 2021). For DCM extractions, a similar procedure was used. Both organic extracts were then stored in bottles in a refrigerator at 4°C until use.

Preparation of different levels of extract concentrations (LECs) from dry extract

Various plant extract concentrations were prepared, mostly according to Gitahi et al. (2021). The dry extracts were diluted with ETOAc and DCM at a concentration of 1 g/ml, and this was called the "stock solution" (100% w/v concentration). The plant extract concentrations used were 25, 50, 75, and 100% (w/v), which were prepared differently. For the 25% (w/v) plant extract concentration, 1 mL of the stock solution was diluted with 3 mL of solvent to produce 4 mL; for the 50% (w/v) plant extract concentration, 2 mL of the stock solution was mixed with 2 mL of solvent to make 4 mL; and for the 75% (w/v) plant extract concentration, 1 mL of solvent was added to 3 mL of the stock solution to make 4 mL (Table 1)

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Table 1. Preparation of solvent extracts (SEs) protocols for determination of the toxicity activities of *N. tabacum* leaf and *J. curcas* seed on *S. zeamais.*

Treated area	Stock solution (ml)	Solvent (ml)
25% PE (w/v)	1	3
50% PE (w/v)	2	2
75% PE (w/v)	3	1
100% PE (w/v)	4	0
Malathion dust 5%	Pc	

Pc=Positive control, PE= plant extract (w/v).
Source: Authors.

Mass rearing of the test insects

The maize grain used in the experiment (BH-661 hybrid maize) was obtained from the Bako National Maize Research Centre and was cleaned and disinfested by being placed in a deep freezer for two weeks at temperatures ranging from 0 to -20°C to remove internal infestations. It was kept for two more weeks in experimental settings for acclimatization by Hiruy and Getu (2020). Initial stock (weevils) was obtained from infested maize purchased from the local market. They were cultured on clean and disinfested maize grain stored in eight 1.5-kg-capacity plastic jars covered with muslin cloth. This was to allow for aeration and prevent weevil escape. After two weeks, all weevils, both alive and dead, were removed from each jar, and the jars were kept in the same laboratory setting. Then, after 30 days, 0 to 3-day-old *S. zeamais* progeny were sieved out and used for the experiment.

Toxicity bioassay of the plants' solvent extracts (SEs)

Following Danga et al. (2015) methods, 100 g of disinfected maize grains were added to 250 cm^3 capacity glass jars with brass screen lids that allow ventilation. Three different rates of each extract (4, 8, and 12 ml) were added to each jar from the four levels of extract concentrations (LECs): 25, 50, 75, and 100%. The jar contents were shaken thoroughly for 5 min to ensure uniform distribution of the solution over the grain surface. The treated grains were then left for 36 h for ETOAc and 24 h for DCM to allow the solvents to completely evaporate before the bioassay. The effect of the solvents (ETOAc and DCM) without a bioassay (the untreated control) was compared to Malathion 5% dust at a recommended rate of 0.05 g/100 g of maize grains (standard check).

Toxicity bioassay of the plants' powder treatments (PTs)

Disinfested maize grains (100 g) were added to 250 cm^3 capacity glass jars with brass screen lids that allow ventilation, and three different rates of each botanical (4, 8, and 12 g) were weighed and added to the grain in each glass jar and shaken well to ensure even distribution (Danga et al., 2015). Following that for both OEs and PTs, 25 pairs of unsexed, laboratory-reared (0–3-day-old) *S. zeamais* were introduced into each jar at the rate of one weevil per 2 g of maize seeds (1:2 g; 50 weevils per 100 g of maize). The jars were kept in the laboratory. When the insects did not respond to a sharp pin inserted into the abdomen, it was determined that they were dead (Ileke and Bulus, 2012).

The effect of SEs and PTs on adult mortality

The mortality data of SEs or PTs-treated jars were collected at 1, 3, 5, and 7 days after treatment application, based on Kidane (2011). Dead adults (*S. zeamais*) were removed and counted during each assessment from each jar. During each assessment day, alive *S. zeamais* returned to their respective colonies.

$$\% \text{ Mortality} = \frac{\text{Number of dead weevils}}{\text{Total number of weevils}} \times 100$$

The effects of SEs and PTs on the emergence of *S. zamias*

On the 14th day of treatment, all the dead and alive *S. zamias* were sieved, counted, and discarded. After that, the grains were placed back in their respective jars and kept under the same experimental conditions for progenies' emergence. F_1 progenies were monitored, counted, and removed every two days for 28 days. Percentage reductions in *S. zamias* emergence or inhibition rate (% IR) were determined using the following formula as adopted by previous researchers (Aboalola et al., 2020).

$$\% \text{ IR} = \frac{(C_n - T_n)}{C_n} \times 100$$

where C_n is the number of newly emerged insects in the untreated check (control) jars and T_n is the number of newly emerged insects in the treatments.

The effects of SEs and PTs on grain weight loss

At the end of the experiment, after 42 days, the percentages of grain damage, weight loss, and germination percentage were calculated. To estimate the percentage of weight loss, 100 maize grains were randomly taken from each jar. Based on the count and weigh method described by Hiruy and Getu (2020), the percentage of weight loss was calculated by counting and weighing the damaged and undamaged grains.

$$\% \text{ Weight loss} = \frac{(W_u \times N_d) - (W_d \times N_u)}{W_u \times (N_d + N_u)} \times 100$$

Table 2. Adult mortality of *S. zeamais* on exposure to *N. tabacum* leaf and *J. curcas* seed SEs of ETOAc.

Botanicals	LEC (%)	Dose (ml)	Mean					
			1day	3days	5days	7 days	14 days	Total
N. tabacum	25	4	16±0^{b}	8±0^{b}	22±0^{fg}	12±0^{bcd}	5.3±1^{ab}	63.3±1^{b}
		8	16±0^{b}	10±0^{bc}	22±0^{fg}	14±0^{bcd}	4±0^{ab}	66±0^{bc}
		12	18±0^{cd}	12±0^{cd}	20±0^{def}	10.7±2^{bcd}	7.3±2^{ab}	68±0^{cd}
	50	4	18±0^{cd}	14±0de	20±0^{def}	12±0^{bcd}	6±2^{ab}	70±0de
		8	18±0^{cd}	14±0de	20±0^{def}	12.7±1^{bcd}	7.3±1^{ab}	72±0^{fgh}
		12	18±0^{cd}	15.3±1^{e}	20.7±1^{ef}	12±1^{bcd}	7.3±1^{ab}	73.3±1^{fgh}
	75	4	18.7±1de	16.7±1^{ef}	19.3±1^{def}	10±1^{bc}	10±1^{b}	74.7±1^{hi}
		8	20±0^{ef}	20.7±1^{gh}	18±0^{cde}	10.7±1^{bcd}	9.3±1^{ab}	78.7±1^{jk}
		12	20±0^{ef}	22±0^{hi}	18±0^{cde}	11.3±1^{bcd}	9.3±1^{ab}	80.7±1^{kl}
	100	4	20.7±1^{fg}	24±1^{ij}	17.3±1^{bcd}	17.3±2^{d}	4.7±2^{ab}	84±1^{lm}
		8	22±0^{gh}	27.3±1^{k}	16±0^{bc}	18±2^{cd}	4±2^{ab}	87.3±1^{mn}
		12	22.7±1^{h}	30.7±1^{l}	15.3±1^{bc}	19.3±1^{d}	2.7±1^{ab}	90.7±1no
J. curcas	25	4	16±0^{b}	8.7±1^{b}	22±0^{fg}	12.7±1^{bcd}	5.3±1^{ab}	64.7±1^{bc}
		8	16.7±1^{bc}	10±1^{bc}	21.3±1^{fg}	10.7±2^{bcd}	7.3±2^{ab}	66±1^{bc}
		12	18±0^{cd}	12±0^{cd}	18±0^{cde}	16±1^{cd}	4±1^{ab}	68±0^{cd}
	50	4	18±0^{cd}	14±0de	20±0^{def}	10±0^{bc}	8.7±1^{ab}	70.7±1^{def}
		8	18±0^{cd}	14±0de	20±0^{def}	12.7±1^{bcd}	7.3±1^{ab}	72±0^{fgh}
		12	18±0^{cd}	16±0^{ef}	20±0^{def}	14±2^{bcd}	6±2^{ab}	74±0^{ghi}
	75	4	20±0^{ef}	18.7±1^{fg}	18±0^{cde}	13.3±2^{bcd}	6.7±2^{ab}	76.7±1^{ij}
		8	20±0^{ef}	22±0^{hi}	18±0^{cde}	17.3±2^{cd}	3.3±2^{ab}	80.7±1^{kl}
		12	20±0^{ef}	22±0^{hi}	18±0^{cde}	15.3±3^{cd}	6±3^{ab}	81.3±1^{kl}
	100	4	22±0^{gh}	26±0^{jk}	16±0^{bc}	16.7±2^{cd}	6.7±1^{ab}	87.3±1^{mn}
		8	22±0^{gh}	28.7±1^{kl}	16±0^{bc}	19.3±1^{d}	2.7±1^{ab}	88.7±1no
		12	23.3±1^{h}	31.3±1^{l}	14.7±1^{b}	17.3±1^{d}	5.3±1^{ab}	92±1^{n}
UC		0	0±0.0^{a}	4.7±0^{a}	5.3±0.0^{g}	6±0.0^{ab}	14±0.0^{b}	30±0^{a}
M5%		0.05	100±0^{g}	0±0^{a}	0±0.0^{a}	0±0.0^{a}	0±0.0^{a}	100^{o}±0
Total			21.6±2	17.1±1	17.5±1	13.3±1	5.87±0	75.2±2
p≤values			0.001	0.001	0.001	0.001	0.001	0.001
LSD0.05			26.1	14.25	7.33	3.64	2.8	13.60
CV%			3.85	5.0	3.41	8.13	39.7	1.79

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test. LEC= Level of extraction concentration, UC= untreated control (ETOAc).
Source: Authors

where Wu = Weight of undamaged grains, Nu = Number of undamaged grains, Wd = Weight of damaged grains, and Nd = Number of damaged grains.

The effects of SEs and PTs on grain damage

Counting methods were used to calculate the grain damage percentage. One hundred grains were randomly selected from each maize sample. The number of damaged grains and undamaged grains was counted for holes or insect burrows using a hand lens. The percentage of grain damage was then calculated as follows (Shiferaw, 2011):

$$\% \text{ damaged grains} = \frac{\text{No. of damaged grains}}{\text{Total No. of grains}} \times 100$$

The effects of PTs on grain germination

The experiment was completed with a germination test after data on many factors were collected. Twenty grains were randomly chosen from both the treatment and control groups for the germination test. These grains were then placed one by one in each sterilized Petri dish containing moistened filter paper (Whatman No. 1) and kept at room temperature around 20 to 22°C (68-72°F). Each treatment was repeated three times. Untreated seeds served as the control. After seven days, the number of seedlings that emerged from each Petri dish was counted and recorded. The percentage of germination was calculated using the formula (Talská et al., 2020).

$$\text{Viability index (\%)} = \frac{N_G}{T_G} \times 100$$

where N_G = number of grains germinated and T_G = total number of grains tested in each Petri dish.

Statistical analysis

Data on percentage of adult mortality, grain weight loss, and grain damage were angularly transformed (arcsine √proportion) to reduce variance heterogeneity, but data on F_1 progeny emergence and percentage of seed germination were square root transformed. The transformed data were examined using the Statistical Package for Social Sciences (SPSS, 2016). A one-way ANOVA was used to perform inferential statistics on this data, followed by Tukey's post hoc test for separation and pairwise mean comparisons. $P < 0.005$ was used to indicate that there was a significant difference between the treatment groups. Back transformed data are shown in the tables

RESULTS

Effects of *N. tabacum* leaf and *J. curcas* seed SEs on the mortality of *S. zeamais*

Tables 2 and 3 show the mortality of *S. zeamais* caused by *N. tabacum* leaf and *J. curcas* seed organic extracts at different concentration levels (LECs) and application dosages for 14 days of exposure. The treatments used in these trials resulted in *S. zeamais* mortality beginning on the first day of treatment. *J. curcas* seed organic extracts of ethyl acetate (ETOAc) in 100% LEC at a dosage of 12 ml showed 92% mean mortality on the 14th day of exposure, while the lowest mortality of 63.3% was recorded from *N. tabacum* leaf extract of ETOAc in 25% LEC at a dose of 4 ml (Table 2). Moreover, all dosages of *J. curcas* seed and *N. tabacum* leaf SEs of ETOAc at different LECs applied were significantly ($p<0.05$) different in *S. zeamais* mortality from the untreated control. Only the chemical insecticide Malathion (5% dust) at a rate of 2 g caused 100% weevil mortality on the first day of treatment application (Tables 2 and 3).

N. tabacum leaf and *J. curcas* seed of DCM SEs in 50, 75, and 100% LECs at all dosages were the most effective, causing 100% mean mortality (Table 3). All LECs were effective insecticides. *S. zeamais* mortality was closely related to each extract concentration level; increases in treatment dose and exposure time were linked to a significant increase in cumulative insect mortality.

Following the 14-day powder treatment, a different mortality percentage was recorded. The least toxic dose of *N. tabacum* leaf powder resulted in only 38% mortality in *S. zeamais* (Table 7). Comparing the toxicity of leaf/seed PTs and SEs from the same plant revealed that SEs were substantially more harmful to test insects than the former (Tables 2, 3, and 5). In the untreated control, test insects were alive for the duration of the study, with only 30% mortality (Tables 2 and 3).

Tables 4 and 5 show the mean emergence of *S. zeamais* after 28 days of exposure to *N. tabacum* leaf and *J. curcas* seed SEs at different LECs. There was no emergence of *S. zeamais* in maize grains treated with Malathion 5% dust at 2 g. *N. tabacum* leaf SEs of ETOAc had the highest emergence (62±0.0) of *S. zeamais,* in 25% LEC at a dose of 4 ml, while it had the lowest emergence (18.3±0.7) of *S. zeamais* in 100% LEC at a dose of 12 ml. *N. tabacum* leaf SEs of DCM caused the highest emergence (60.3±0.3) of *S. zeamais* in 25% LEC at the dose of 4 ml, but it induced the lowest emergence (16±0.0) of *S. zeamais* in 100% LEC at the dose of 12 ml (Table 5). The results of this study showed that *N. tobacco* leaf SEs of ETOAc had comparatively higher emergence of *S. zeamais*, since about 62% of *S. zeamais* emergence was recorded in 25% LEC at a dose of 4 ml after 28 days of treatment (Tables 4 and 5).

On the other hand, the number of emerging *S. zeamais* in untreated grains was far too high (134). *J. curcas* seed SEs of ETOAc in 100% LEC at a dose of 12 ml inhibited 88.56% of the emergence of *S. zeamais*, *N. tabacum* leaf SEs of DCM, and *J. curcas* seed SEs of ETOAc in 100% LEC at a rate of 12 ml suppressed 87.3% of the emergence of *S. zeamais*. Malathion 5% dust at 2 g inhibited 100% of *S. zeamais* adult emergence (Table 5). Table 6 shows the mean percentage of weight loss and grain damage to maize grains due to *S. zeamais.* This is influenced by *N. tabacum* leaf and *J. curcas* seed SEs at various LECs and application dosages at the end of the 42-day experiment. The grain weight loss treated with *J. curcas* seed SEs of DCM was lower than that of grains treated with *N. tabacum* leaf SEs of ETOAc and the untreated control. *N. tabacum* leaf SEs of ETOAc in 25% LEC at a dose of 4 ml caused the most grain damage, followed by *J. curcas* seed SEs of ETOAc in 25% LEC at a dose of 4 ml, then *N. tabacum* leaf SEs of ETOAc in 25% LEC at a dose of 8 ml, and *N. tabacum* leaf SEs of ETOAc in 25% LEC at a dose of 12 ml (30.0, 29.2, 28.4, and 26.93%, respectively) (Table 6). Similarly, *J. curcas* seed SEs of DCM in 100% LEC gave pronounced antifeedant effects with significantly lower grain damage of 12.4% at a dose of 4 ml, 9.73% at a dose of 8 ml, and 6.8% at a dose of 12 ml when compared with untreated

Table 3. Percent adult mortality of *S. zeamais* on exposure to *N. tabacum* leaf and *J. curcas* seed SEs of DCM.

Botanicals	LEC (%)	Dose (ml)	Mean					
			1 day	3 days	5 days	7 days	14 days	Total
N. tabacum	25	4	13.3±1^{b}	14.7±1^{c}	16.7±1^{c}	14±0^{c}	11.3±3^{c}	68.7±1^{b}
		8	13.3±3^{b}	14±0^{c}	18±0^{cd}	19.3±1^{bc}	18.7±1^{d}	82±0^{c}
		12	19.3±1^{cd}	14±0^{c}	18±0^{cd}	22±0^{c}	20±0^{d}	93.3±1^{d}
	50	4	20±2^{d}	18.7±1^{c}	22.7±1^{d}	20±0^{bc}	18.7±1^{d}	100±0^{f}
		8	18±0^{bcd}	20±0^{c}	18±0^{cd}	22±0^{c}	22±0^{d}	100±0^{f}
		12	22±0^{d}	18±0^{c}	18±0^{cd}	20±0^{bc}	22±0^{d}	100±0^{f}
	75	4	22±0^{d}	18±0^{c}	22±0^{d}	20±0^{bc}	18±0^{d}	100±0^{f}
		8	20±0^{d}	18±0^{c}	18±0^{cd}	22±0^{c}	22±0^{d}	100±0^{f}
		12	20.7±1^{d}	18.7±1^{c}	19.3±1^{cd}	20.7±1^{c}	20.7±1^{d}	100±0^{f}
	100	4	18.7±1^{bcd}	18±0^{c}	20.7±1^{cd}	20.7±1^{c}	22±0^{d}	100±0^{f}
		8	19±1^{bcd}	18±0^{c}	20±1^{cd}	21±1^{c}	22±0^{d}	100±0^{f}
		12	21±1^{d}	16±1^{c}	19±1^{cd}	22±0^{c}	22±0^{d}	100±0^{f}
J. curcas	25	4	14±1^{bc}	14±1^{c}	18±1^{c}	18±1^{bc}	18±0^{d}	82±1^{c}
		8	17.3±0^{bcd}	14±0^{c}	18±0^{cd}	20.7±1^{bc}	18±0^{d}	88±0^{c}
		12	19.3±1^{cd}	18.7±3^{c}	16.7±1^{cd}	20.7±1^{c}	21.3±1	96.7±0^{e}
	50	4	19±1^{bcd}	21±1^{c}	18±0^{cd}	21±1^{c}	21±1^{d}	100±0^{f}
		8	18±0^{bcd}	21±1^{c}	18±0^{cd}	21±1^{c}	22±0^{d}	100±0^{f}
		12	20.7±1^{d}	19.3±1^{c}	20.7±1^{cd}	20±0^{bc}	19.3±1^{d}	100±0^{f}
	75	4	19.3±1^{cd}	18.7±1^{c}	22±0^{d}	19.3±1^{bc}	20.7±1^{d}	100±0^{f}
		8	22±0^{d}	19.3±1^{c}	20.7±1^{cd}	19.3±1^{bc}	18.7±1^{d}	100±0^{f}
		12	21±1^{d}	19±1^{c}	20±1^{cd}	21±2^{c}	19±1^{d}	100±0^{f}
	100	4	21±1^{d}	19±1^{c}	20±1^{cd}	20±1^{bc}	20±1^{d}	100±0^{f}
		8	22±0^{d}	14±4^{c}	22±0^{d}	22±2^{c}	20±1^{d}	100±0^{f}
		12	21.3±1^{d}	16±2^{c}	19.3±1^{cd}	21.3±1^{c}	22±0^{d}	100±0^{f}
UC		0	0±0^{a}	4.7±0^{b}	5.3±0^{b}	6±0^{b}	14±0^{b}	30±0^{a}
M5%		0.05	100±0^{a}	0±0^{a}	0±0^{a}	0±0^{c}	0±0^{d}	100^{f}±0
Total			21.9±2	16.3±1	18.3±1	19.2±1	18.8±1	94.4±2
p <values			0.08	0.08	0.06	0.02	0.06	0.08
LSD0.05			22.68	8.43	8.7	12.73	9.25	24.1
CV%			4.15	4.43	2.84	3.18	2.94	0.21

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey's test. LEC= Level of extraction concentration, UC= Untreated Control (DCM).
Source: Authors.

controls (66.27%). In conclusion, *J. curcas* seed powder at 12 g and its SEs with ETOAc and DCM in 100% LEC at a dose of 12 ml experienced the least grain weight loss (1.36, 0.79 and 0.64%, respectively) (Tables 6 and 9). Untreated controls had the greatest grain weight loss (5.88%).

The efficacy of *N. tabacum* leaf and *J. curcas* seed PTs against *S. zeamais*

Table 7 shows the mean percent mortality of *S. zeamais* on maize grains treated with *N. tabacum* leaf and *J. curcas* seed PTs. The results indicated that the PTs of

Table 4. Mean number of adult *S. zeamais* emerging and percent protection in *N. tabacum* leaf and *J. curcas* seed SEs of ETOAc.

Botanicals	LEC (%)	Dose (ml)	Mean number of F_1 progeny emerged			% IR
			14 days	28 days	Total	
N. tabacum	25	4	32 ± 0.0^{q}	30 ± 0.0^{p}	62 ± 0.0^{p}	54.73 ± 0.25^{b}
		8	30 ± 0.0^{p}	28 ± 0.0^{op}	58 ± 0.0^{no}	56.97 ± 0.25^{c}
		12	28 ± 0.0^{no}	27 ± 0.0^{no}	55 ± 0.0^{mn}	59.7 ± 0.00^{d}
	50	4	27.7 ± 0.3^{mn}	26 ± 0.0^{mn}	53.7 ± 0.3^{lm}	62.19 ± 0.5^{de}
		8	25 ± 0.0^{kl}	24 ± 0.0^{kl}	49 ± 0.0^{jk}	64.18 ± 0.00^{ef}
		12	24 ± 0.0^{kl}	23 ± 0.0^{jk}	47 ± 0.0^{ij}	65.67 ± 0.00^{gh}
	75	4	23 ± 0.0^{ij}	22±0.0ij	45 ± 0.0^{hi}	68.16 ± 0.25^{ij}
		8	20 ± 0.0^{gh}	19 ± 0.0^{gh}	39 ± 0.0^{g}	73.13 ± 0.43^{k}
		12	18 ± 0.0^{f}	17 ± 0.0^{f}	35 ± 0.0^{f}	75.12 ± 0.25^{l}
	100	4	15.7 ± 0.7^{e}	14.7 ± 0.7^{e}	30.3 ± 0.0^{e}	78.61 ± 0.66^{m}
		8	13.5 ± 0.3^{d}	12.5 ± 0.3^{d}	26 ± 0.0^{d}	82.84 ± 0.43^{n}
		12	10.5 ± 0.3^{bc}	9.5 ± 0.3^{bc}	20 ± 0.0^{bc}	86.82 ± 0.5^{p}
J. curcas	25	4	31 ± 0.0^{pq}	29.5 ± 0.3^{pq}	60.5 ± 0.3^{op}	$55.47\pm0.25_{bc}$
		8	29.7 ± 0.3^{op}	27 ± 0.0^{no}	56.7 ± 0.3^{mn}	58.21 ± 0.43^{c}
		12	28 ± 0.0^{no}	27 ± 0.0^{no}	55 ± 0.0^{mn}	60.45 ± 0.43^{c}
	50	4	26 ± 0.0^{lm}	25 ± 0.0^{lm}	51 ± 0.0^{kl}	63.43 ± 0.43^{e}
		8	25 ± 0.0^{kl}	24 ± 0.0^{kl}	49 ± 0.7^{jk}	65.17 ± 0.25^{ef}
		12	23.7 ± 0.3^{kl}	22.7 ± 0.3^{jk}	46.3 ± 0.7^{ij}	66.67 ± 0.5^{fg}
	75	4	21.7 ± 0.3^{hi}	20.7 ± 0.3^{hi}	42.3 ± 0.7^{h}	70.4 ± 0.66^{hi}
		8	18.7 ± 0.3^{fg}	17.7 ± 0.3^{fg}	36.3 ± 0.7^{fg}	74.38 ± 0.25^{j}
		12	17.5 ± 0.3^{f}	16.5 ± 0.3^{f}	34 ± 0.6^{f}	76.87 ± 0.43^{l}
	100	4	14.5 ± 0.3^{de}	13.5 ± 0.3^{de}	28 ± 0.6^{de}	80.85 ± 0.25^{lm}
		8	11.5 ± 0.3^{c}	10.5 ± 0.3^{c}	22 ± 0.6^{c}	85.07 ± 0.43^{n}
		12	9.7 ± 0.3^{b}	8.7 ± 0.3^{b}	18.3 ± 0.7^{b}	88.56 ± 0.5^{o}
UC		0	79.3 ± 3^{e}	54.7 ± 2^{f}	134 ± 2^{g}	0 ± 1.14^{p}
M5%		0.05	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.6^{a}	100 ± 0.00^{a}
Total			20.7±0.9	19.8±1.0	40.5±0.6	68.22±2.02
p ≤values			0.001	0.001	0.001	0.001
LSD0.05			22.88	22.19	25.63	7.41
CV%			3.53	3.72	3.43	4.3

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test. LEC= Level of extraction concentration, UC= Untreated Control (ETOAc).
Source: Authors.

the two different plant species significantly (P<0.05) reduced the number of *S. zeamais*. The lowest concentration of the PTs used in this study (4 g) induced only 38% mortality within 14 days after *S. zeamais* were exposed to the powder. When the powder concentration was increased to 8 g, mortality increased to 53% within 14 days of the mortality study. This percentage of mortality gradually increased to 62% when the powder

Table 5. The mean number of *S. zeamais* emerged and the percent protection in *N. tabacum* leaf and *J. curcas* seed SEs of DCM.

Botanicals	LEC (%)	Dose (ml)	Mean number of F_1 progeny emerged			% IR
			14 days	28 days	Total	
	25	4	31 ± 0.0^{r}	29.3 ± 0.3^{r}	60.3 ± 0.3^{q}	53.7 ± 0.00^{b}
		8	29 ± 0.0^{pqr}	28.3 ± 0.3^{qr}	57.3 ± 0.3^{op}	56.2 ± 0.5^{cd}
		12	27.7 ± 0.3^{op}	26.3 ± 0.3^{opq}	54 ± 0.0^{mn}	58.7 ± 0.25^{ef}
	50	4	26.7 ± 0.3^{mn}	24.7 ± 0.3^{no}	51.3 ± 0.7^{l}	60.2 ± 0.5^{g}
		8	24 ± 0.0^{klm}	24 ± 0.0^{mn}	48 ± 0.0^{k}	62.4 ± 0.5^{h}
		12	23 ± 0.0^{jk}	23 ± 0.0^{lm}	46 ± 0.0^{jk}	64.4 ± 0.00^{hi}
N. tabacum						
	75	4	$2^{2}\pm0.0^{ij}$	21 ± 0.0^{k}	43 ± 0.0^{i}	66.9 ± 0.43^{j}
		8	19 ± 0.0^{gh}	17.3 ± 0.3^{ij}	36.3 ± 0.3^{g}	71.4 ± 0.43^{l}
		12	17 ± 0.0^{f}	16.3 ± 0.3^{hi}	33.3 ± 0.3^{f}	73.9 ± 0.00^{m}
	100	4	14.7 ± 0.7^{de}	13.3 ± 0.3^{fg}	28 ± 1.0^{e}	76.9 ± 0.5^{n}
		8	12.5 ± 0.3^{c}	11 ± 0.0^{de}	23.5 ± 0.3^{d}	80.9 ± 0.5^{o}
		12	9.5 ± 0.3^{b}	8.5 ± 0.3^{bc}	18 ± 0.6^{bc}	85.3 ± 0.5^{pq}
	25	4	30.5 ± 0.3^{qr}	29 ± 0.0^{r}	59.5 ± 0.3^{pq}	54.5 ± 0.25^{bc}
		8	28.7 ± 0.3^{opq}	27 ± 0.0^{pq}	55.7 ± 0.3^{no}	57.5 ± 0.5^{de}
		12	27 ± 0.0^{no}	25.7 ± 0.3^{nop}	52.7 ± 0.3^{lm}	59.0 ± 0.5^{fg}
	50	4	25 ± 0.0^{lm}	23.5 ± 0.5^{mn}	48.5 ± 0.5^{k}	61 ± 0.86^{h}
		8	24 ± 0.0^{lm}	23 ± 0.0^{mn}	47 ± 0.0^{jk}	63 ± 0.5^{hi}
		12	23.3 ± 0.7^{kl}	22 ± 0.0^{kl}	45.3 ± 0.7^{ij}	64 ± 0.5^{ij}
J. curcas						
	75	4	20.7 ± 0.3^{hi}	18.7 ± 0.3^{j}	39.3 ± 0.7^{h}	64 ± 0.5^{k}
		8	17.3 ± 0.3^{fg}	16.7 ± 0.3^{hi}	34 ± 0.0^{fg}	73 ± 0.00^{lm}
		12	16.5 ± 0.3^{ef}	15 ± 0.0^{gh}	31.5 ± 0.3^{f}	75 ± 0.5^{m}
	100	4	13.5 ± 0.3^{cd}	12 ± 0.0^{ef}	25.5 ± 0.3^{de}	79 ± 0.5^{no}
		8	10.5 ± 0.3^{b}	9.5 ± 0.3^{cd}	20 ± 0.6^{c}	83.3 ± 0.5^{p}
		12	8.7 ± 0.3^{b}	7.3 ± 0.3^{b}	16 ± 0.0^{b}	87.3 ± 0.86^{q}
UC		0	79.3 ± 3^{e}	54.7 ± 2^{f}	134 ± 2^{g}	0 ± 1.14^{a}
M5%		0.05	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{a}	100 ± 0^{a}
Total			19.9±1.0	18.8±1.0	38.5±1.9	66.9±2
p ≤values			0.001	0.001	0.001	0.001
LSD0.05			9.94	10.35	19.65	29.96
CV%			3.99	4.02	3.71	6.00

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test., M5%= Malathion 5% dust, LEC= Level of extraction concentration, UC= Untreated Control (DCM).
Source: Authors.

concentration increased to 12 g. Only 47.3% of the *S. zeamais* mortality was recorded when the insects were exposed to the *J. curcas* seed powder at the study's lowest concentration (4 g) perished within 14 days. The 12 g powder concentration showed a mortality of 65.3% within 14 days of insects' exposure to the powder, while the 8 g powder concentration killed 56.7% of *S. zeamais* within the same 14 days (Table 7).

The percentage mortality recorded at 4, 8, and 12 g powder concentrations was significantly different for the

Table 6. The mean number of grain damage and grain weight loss caused by *S. zeamais* on maize grains treated with *N. tabacum* leaf and *J. curcas* seed SEs of ETOAc/DCM.

Botanicals	LEC (%)	Dose (ml)	Mean			
			ETOAc		DCM	
			% GD	% WL	% GD	% WL
		4	30±0.13^{u}	2.8±0.01^{t}	30±0.23^{t}	2.81±0.02^{t}
	25	8	28.4±0.00st	2.66±0.00^{rs}	28.4±0.23^{rs}	2.66±0.02^{rs}
		12	26.93±0.13^{qr}	2.52±0.01^{pqr}	26.67±0.27^{pq}	2.5±0.02^{pq}
		4	25.87±0.13^{opq}	2.43±0.00^{nop}	25.33±0.13^{op}	2.37±0.01^{op}
	50	8	24.53±0.13^{mno}	2.3±0.02^{lmk}	23.73±0.13^{mn}	2.22±0.01^{mn}
		12	23.2±23^{lm}	2.18±0.01^{kl}	22.4±0.23^{lm}	2.1±0.02^{lm}
N. tabacum						
		4	21.73±27^{jk}	2.04±0.01^{j}	20.8±0.23^{jk}	1.95±0.02^{jk}
	75	8	19.33±35^{i}	1.81±0.01^{i}	17.87±0.35^{i}	1.68±0.04^{i}
		12	17.2±23^{gh}	1.61±0.02^{gh}	16±0.23^{gh}	1.5±0.02^{gh}
		4	15.2±46^{f}	1.43±0.01^{f}	14±0.46^{f}	1.31±0.04^{f}
	100	8	12.53±0.13^{de}	1.17±0.00^{de}	11.07±0.27^{de}	1.04±0.03^{de}
		12	10±46^{c}	0.94±0.02^{c}	8.4±0.46^{c}	0.79±0.04^{c}
		4	29.2±0.00^{ty}	2.74±0.02st	29.2±0.23st	2.74±0.02st
	25	8	27.6±23^{rs}	2.59±0.04^{qr}	27.6±0.23^{rq}	2.59±0.02^{qr}
		12	26.27±013^{pqr}	2.47±0.02^{opq}	25.87±0.13^{op}	2.43^{op}±0.01^{op}
		4	25.07±0.13^{nop}	2.35±0.03^{mno}	24.4±0.23^{no}	2.29±0.02^{no}
	50	8	24±0.00^{mn}	2.25±0.01^{lm}	23.2±0.00^{mn}	2.18±0.00^{mn}
		12	22.4±0.23^{kl}	2.1±0.01^{jk}	21.6±0.23^{kl}	2.03±0.02^{kl}
J. curcas						
		4	20.93±0.27^{j}	1.96±0.04^{j}	19.6±0.23^{j}	1.84±0.04^{ij}
	75	8	18.27±0.13^{hi}	1.72±0.01^{hi}	16.8±0.23^{hi}	1.58±0.02^{hi}
		12	16.53±0.13^{fg}	1.55±0.04^{fg}	15.2±0.46^{fg}	1.43±0.02^{fg}
		4	13.73±0.48^{e}	1.29±0.04^{fg}	12.4±0.237^{e}	1.16±0.04^{e}
	100	8	11.33±0.27^{cd}	1.06±0.03^{cd}	9.73±0.46^{cd}	0.91±0.03^{cd}
		12	8.4±0.46^{b}	0.79±0.04^{b}	6.8±0.23^{b}	0.64±0.04^{b}
UC		0	66.27±0.00^{v}	5.88±0.00^{u}	66.27±0.00^{u}	5.88±0.00^{u}
M5%		0.05	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}	0±0.00^{a}
Total			20.55±0.89	1.93±08	19.59±0.91	1.84±0.09
p ≤values			0.001	0.001	0.001	0.001
LSD0.05			24.89	24.77	23.85	23.94
CV%			3.78	3.8	4.45	4.49

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test. LEC= Level of extraction concentration, GD= grain damage, WL= weight loss, UC= Untreated Control (ETOAc/DCM), M5%= Malathion 5% dust.
Source: Authors

duration of 1, 3, 5, 7, and 14 days following *S. zeamais'* introduction into the jars ($p<0.05$) (Table 7). Both *N. tabacum* leaf powder and *J. curcas* seed PTs were found to be very effective in insect mortality and adult

Table 7. The mean number of mortality of *S. zeamais* on maize grains treated with *N. tabacum* leaf and *J. curcas* seed PTs.

Botanicals	Dose (g)	Means					
		1 day	3 days	5 days	7 days	14 days	Total
N. tabacum	4	2±0^{ab}	4.7±1^{ab}	6.7±1^{ab}	8±1^{ab}	16.7±1^{ab}	38±1^{b}
	8	2±0^{ba}	12.7±3^{bc}	9.3±3^{c}	13.3±4^{c}	16±3^{ab}	53.3±1^{cd}
	12	0.7±1^{c}	16.7±2^{c}	12.7±4^{c}	15.3±2^{c}	16.7±6^{c}	62±2^{ef}
J. curcas	4	1.3±1^{abc}	7.3±1^{abc}	11.3±1^{c}	12.7±2^{c}	14.7±3^{c}	47.3±3^{c}
	8	0.7±1^{c}	15.3±5^{c}	8±2^{ab}	12.7±5^{c}	20±4^{c}	56.7±1de
	12	2±0^{c}	16.7±1^{c}	9.3±1^{c}	18±5^{c}	19.3±5^{c}	65.3±1^{f}
UC	0	0±0^{ab}	4.7±1^{ab}	5.3±1^{ab}	6±0^{ab}	14±2^{ab}	30±0^{a}
M5%	0.05	100±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	0±0^{a}	100±0^{g}
Total		13.6±7	9.8±1	7.8±1	10.8±1	14.7±2	56.6±4
p ≤values		0.001	0.001	0.006	0.003	0.019	0.001
LSD0.05		41.6	11.4	8.8	12	16.8	25.7
CV%		84.8	34.1	35.8	32.5	36.3	6.9

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test. UC= Untreated Control, M5%= Malathion 5% dust
Source: Authors

Table 8. The mean number of F_1 progeny of *S. zeamais* produced and the percent protection of *N. tabacum* leaf and *J. curcas* seed PTs of maize grains.

Botanicals	Dose (g)	Mean number of F_1 progeny emerged			
		14 days	28 days	Total	% IR
N. tabacum	4	53.3±1^{d}	52.7±3^{ef}	106±2^{f}	20.9±2^{b}
	8	50±4^{cd}	36.7±3^{cd}	86.7±6de	35.32±5^{cd}
	12	35±3^{b}	32±1^{bc}	67±2^{bc}	50±2^{ef}
J. curcas	4	50.7±1^{cd}	44±2de	94.7±2^{ef}	29.35±2^{bc}
	8	41.3±2^{bc}	35±1^{bcd}	76.3±1^{cd}	43.03±1de
	12	30.7±2^{b}	27±2^{b}	57.7±1^{b}	56.97±1^{f}
UC	0	79.3±3^{e}	54.7±2^{f}	134±2^{g}	0±1^{a}
M%	0.05	0±0^{a}	0±0^{a}	0±0^{a}	100±0^{g}
Total		42.5±5	35.3±3	77.8±8	41.95±6
LSD0.05		9.69	8.8	12.	12.
p ≤values		0.001	0.001	0.001	0.001
CV%		14.01	13	3.8	67.43

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA (P ≤ 0.05) followed by Tukey`s test. UC= Untreated Control, M5%= Malathion 5% dust.
Source: Authors.

emergence. The toxicity of these plant powders increased as the dosage and duration of exposure increased. *J. curcas* seed PTs were more toxic than *N. tabacum* leaf PTs to insects (Table 7).

Table 8 shows the mean number of F_1 progeny of *S. zeamais* that emerged from *N. tabacum* leaf and *J. curcas* seed PTs at different concentration doses. When maize was treated with *N. tabacum* leaf PTs at doses of 4 to 12 g, 106 to 67 adult *S. zeamais* were produced. Conversely, when maize was treated with *J. curcas* seed

Table 9. Mean numbers of percent grain damage, weight loss, and germination caused by *S. zeamais* on maize grains treated with *N. tabacum* leaf and *J. curcas* seed powders.

Botanicals	Dose (g)	Mean		
		% GD	% WL	% Germination
N. tabacum	4	42.67±0.67^{d}	4.35±4.11^{f}	57.6±0.8^{b}
	8	24.53±4.21^{c}	2.71±0.11^{f}	75.87±4.2^{c}
	12	14.4±2.27^{bc}	1.67±0.01^{bc}	85.87±2.2^{cd}
J. curcas	4	27.47±4.5^{c}	3.36±0.41^{c}	72.8±4.2^{c}
	8	24.53±4.39^{c}	2.31±0.3^{c}	75.9±4.4^{c}
	12	9.6±1.4^{ab}	1.36±0.27^{ab}	90.8±1.4^{de}
UC	0	66.27±0^{e}	5.88±0.1^{e}	34.13±0.27^{a}
M5%	0.05	0±0.27^{e}	0±0.00^{a}	100±0.0^{e}
Total		26.18±00	2.71±0.36	74.12±4.1
p≤values		0.001	0.001	0.001
LSD0.05		6.88	8.78	6.82
CV%		27.78	17.49	7.57

Values followed by the same superscript within the same column are not significantly different by one-way ANOVA **(P ≤** 0.05) followed by Tukey`s test. GD= grain damage, WL= weight loss, UC= Untreated Control. M5%= Malathion 5% dust.
Source: Authors.

PTs, 94.7 to 57.7 adult *S. zeamais* were produced using *N. tabacum* leaf PTs (Table 8). At high doses, both *N. tabacum* leaf and *J. curcas* seed PTs significantly reduced *S*. *zeamais* emergence.

Compared to grains treated with *J. curcas* seed powder, grains treated with *N. tabacum* leaf powder had the highest number of F_1 progeny on the 14th day. At a dose of 4 to 12 g, 53.3 to 35 adults of *S. zeamais* were recorded. At the same dose, however, 50.7 to 30.7 adults of *S. zeamais* were found. For the next 28 days, the outcome followed the same pattern. The untried (control) group produced the most F_1 *S. zeamais* (134), followed by *N. tabacum* leaf powder at a dose of 4 g (106) adults (Table 8).

When the efficacy of the two plants was compared after 28 days, the maize grains treated with *J. curcas* seed powder produced the fewest F_1 *S. zeamais*. In general, *N. tabacum* leaf powder was less effective than *J. curcas* seed powder.

Table 9 shows the mean percentage of grain damage, weight loss, and germination caused by *S. zeamais* on maize grains treated with *N. tabacum* leaf and *J. curcas* seed powders at different concentration doses. The assessment of grain damage showed that grains treated with *N. tabacum* leaf powder were most damaged (14.4-42.67%), whereas the least damaged grains were those treated with *J. curcas* seed powder (9.6-27.47%).

After the untreated control, the percentage of seed weight loss of maize grain treated with *N. tabacum* leaf powder (4.35% at 4 g treatment and 1.67% at 12 g treatment) was the second highest number of weight losses. On the other hand, this result significantly differed from the grain weight loss recorded on grains treated with *J. curcas* seed powder (3.36% at 4 g treatment and 1.36% at 12 g treatment). Both results were statistically different from untreated seeds (the control), which recorded a weight loss of 5.88%.

The percentage germination of maize grains treated with *J. curcas* seed powder (72.8% at 4 g, 75.9 at 8 g, and 90.8% at 12 g) was significantly higher than seeds treated with *N. tabacum* leaf powder at all dose rates (57.6% at 4 g, 75.87 at 8 g, and 85.87% at 12 g). *N. tabacum* leaf and *J. curcas* seed powders had a significant effect on maize seeds germination compared with the control group (Table 9).

DISCUSSION

The current study showed that both leaf and seed solvent extracts of DCM and ETOAc had the strongest protective capacity against insects. They caused mortality and a complete reduction of *S. zeamais* F_1 progeny. These results agree with Kavallieratos et al. (2023), who reported that in grains treated with *J. curcas* L. oil (Euphorbiaceae), there was a lower emergence of *S. oryzae* and *Ephestia küehniella* Zeller (Lepidoptera: Pyralidae) compared with untreated grains. The high

concentration of phenol ester in the seeds may explain the superior biocidal activity of *J. curcas* oil extracts (Saosoong et al., 2016). Application of the extracts directly was more efficient since extract molecules are absorbed by insects' integuments, affecting their central nervous system and leading to their death (Ubulom et al., 2021).

Nicotine quickly kills insects, causing intense tremors, convulsions, and paralysis. It competes with the major neurotransmitter, acetylcholine, by binding to acetylcholine receptors at nerve synapses and causing uncontrolled nerve firing (Kanmani et al., 2021). Nicotine, as an alkaloid, disrupts biological membranes, malfunctions internal organs and metabolism, redox imbalance, disruptions in insect development and reproduction, and inhibition of food intake (El-Wakeil, 2013; Akbar et al., 2022).

Grain weight loss is an indicator of insect pest damage during storage (Ngom et al., 2020). A small weight loss of grains was observed when maize was treated with *J. curcas* seed and *N. tobacum* leaf SEs and powder formulations. The plant materials demonstrated excellent protection against maize damage caused by *S. zeamais*. Temitope (2014) found seed SEs of *J. curcas* exhibited antifeedant and grain protectant effects, producing excellent results against *S. zeamais.* Likewise, Viteri-Jumbo et al. (2018) and Idoko and Ileke (2020) showed that sub-lethal doses of essential oil significantly reduced grain damage since oviposition rates were reduced.

This study revealed that the number of *S. zeamais* present was related to maize grain weight losses. This means that because the majority of weevils died shortly after introduction, there was less adult emergence in seeds treated with SEs and powder formulations. In addition, feeding activities were reduced, and thus fewer weight losses were recorded. The present results are in agreement with Gariba et al. (2021) findings in which less progeny and grain weight loss were recorded in maize grains treated with 0.05 and 0.1 g/mL organic extracts of *Lantana camara*, *Hyptis suaveolens, Citrus sinensis*, and *Moringa oleifera*. In addition to their toxicity, the botanicals' chemical constituents may have inhibited weevil feeding on the treated maize grains, thereby protecting the grains from damage (Oboho et al., 2016).

Overall, the results showed that treating maize with *N. tabacum* leaf and *J. curcas* seed powders and SEs at the majority of the investigated dosages reduced the emergence of *S. zeamais*, decreased weight loss, and reduced seed damage. So, because these plant resources are inexpensive, readily available, simple and safe to use, farmers should be encouraged to use them to control *S. zeamais.* They should also protect stored maize.

Conclusion

For a long time, plant materials, crude organic plant extracts, and essential oils have been used to protect crops from insect pests. This is due to the fact that these plant-based products are very promising, generally available, economical, and simple to produce and apply to grains. They are non-toxic to non-target organisms and have a specific mode of action. Based on the findings of the present study, it can be concluded that *N. tabacum* leaves and *J. curcas* seeds SEs of ETOAc and DCM could be used for the control of *S. zeamais* under farmers' storage conditions. Hence, farmers are advised to utilize native plant organic extracts to control *S. zeamais* and safeguard stored maize. Further studies to isolate pure compounds from *N. tabacum* leaf and *J. curcas* seed organic extracts and determine the mode of action are suggested.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of viral hepatitis C screening at Institut Pasteur of Côte d'Ivoire from 2012 to 2022

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The hepatitis C virus (HCV) accounts for 48% of the 1.4 million deaths from viral hepatitis worldwide. The prevalence of HCV was estimated at 5% in Côte d'Ivoire in 2015. The aim is to evaluate screening for the HCV at the Institut Pasteur de Côte d'Ivoire (IPCI) from 2012 to 2022. A retrospective study was carried out to monitor changes in screening for the anti-HCV Ac at the IPCI. The parameters of interest were age, sex and anti-HCV antibody results during the study period. The data collected were recorded in an Excel file and analysed using Rstudio software. According to the results obtained, the positivity rate for males was 5.26% compared with 3.81% for females, and the financial support provided by ROCHE stimulated the screening of a large number of people. The positive rate for viral hepatitis C (4.6%) virtually mirrored the rate of 4.4% obtained in a study of blood donors in Côte d'Ivoire. Finally, this study demonstrated the endemic nature of the HCV in Côte d'Ivoire. Despite financial support to facilitate screening, the seroconversion rate among the population has not shown any increase in the number of people contracting the HCV.

Key words: Hepatitis C virus (HCV), prevalence, proportion of positivity.

INTRODUCTION

Viral hepatitis is a major public health problem. They are considered the seventh leading cause of death worldwide (Ongaro and Negro, 2022). Among them, viral hepatitis C occupies a key position due to the hepatic complications associated with it. It is slow progression from the acute to the cancer stage leading to chronicity in 60 to 80% of cases, followed by cirrhosis in 15 to 30% (Echeverría et al., 2021).

An estimated 71 million people worldwide are living with the chronic hepatitis C virus (HCV) (Dugan et al., 2021). Viral hepatitis C accounts for 48% of the 1.4 million deaths from viral hepatitis worldwide (Ongaro and Negro, 2022).

If HCV-infected people remain undiagnosed and untreated, the HCV mortality burden will continue to rise (Umutesi et al., 2019). Thus, the WHO has proposed the elimination of viral hepatitis C as a public health burden by 2030. To achieve this goal, countries around the world must reach a diagnosis rate of 90%, a treatment rate of 80% and a 75% reduction in the risk

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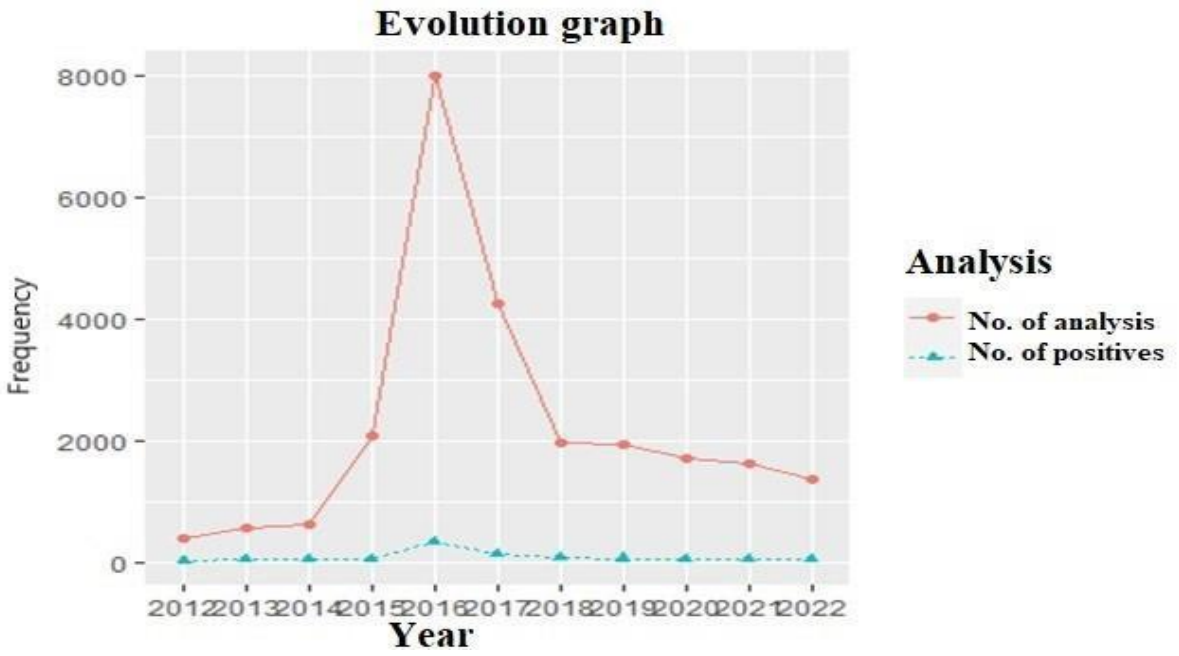

Figure 1. Samples analysed and positivity rates over the years.

(Zhou et al., 2023).

In North Africa, HCV prevalence is very high, especially in Egypt, where the rate can exceed 15%. Central and West Africa are areas of high endemicity, with prevalences in excess of 8% (Kalla et al., 2020).

In West Africa, HCV prevalence varies from country to country. It is 3% in Ghana (Masood and Mahbobeh, 2020), 4.12% in Benin (Kpossou et al., 2021), and 3.8% in Burkina Faso (Meda et al., 2018).

In Côte d'Ivoire, while HCV prevalence was estimated at 5% in 2015 (Enel et al., 2015) very few recent studies have been carried out on this state of affairs unlike in neighboring countries.

The aim of this work is to evaluate HCV screening at the Institut Pasteur de Côte d'Ivoire frequented by the entire Ivorian population over the past 11 years.

MATERIALS AND METHODS

This is a retrospective study, which was carried out at the Bacterial and Viral Serology Unit of the Institut Pasteur de Cote d'Ivoire (IPCI) over the period from 2012 to 2022. The study involved data collected from viral hepatitis screening registers. The parameters of interest were age, sex and anti- HVC antibody results during the study period. After consulting the databases, anti-HVC antibody screening was determined by year, sex, and defined age ranges. A comparative study was then carried out to assess changes in the frequency of HCV screening during the study period.

All these parameters of interest were saved in an Excel file and analyzed using Rstudio software. The comparison of proportions test based on the χ^2 statistic was used to compare two or more different proportions with the prop.test() and pairwise.prop.test() functions, respectively. The test is significant when its p-value is less than 0.05.

RESULTS

Trend in number of samples tested

A total of 24,687 samples were tested for anti-HCV Ac during the study period from 2012 to 2022, and 1,127 were positive, representing a prevalence of 4.6%. 12,973 (52.55%) were male, including 682 positives (5.26%), and 11,714 (47.45%) were female, including 447 positives (3.81%). The sex ratio was 1.11. Age ranged from 1 to 87, with an average of 39.71. We saw an increase in the number of analyses carried out in 2015 and 2016.

This increase is due to the financing of tests by the ROCHE company. This financial support has boosted screening.

Many patients who were ill or wanted to know their status with regard to viral hepatitis C came to our services. The percentage of positive tests for immunological response did not increase. It remains in the same range as in other years. HCV is circulating well in Côte d'Ivoire, but not at a very high level as for hepatitis B virus. A gradual decline in numbers has been observed from 2017 to 2022 (Figure 1).

Figure 2 shows the distribution of the number of samples analyzed from 2012 to 2014 (period before Roche's agreement with the Ivory Coast government), from 2015 to 2017 (period of the agreement), and from

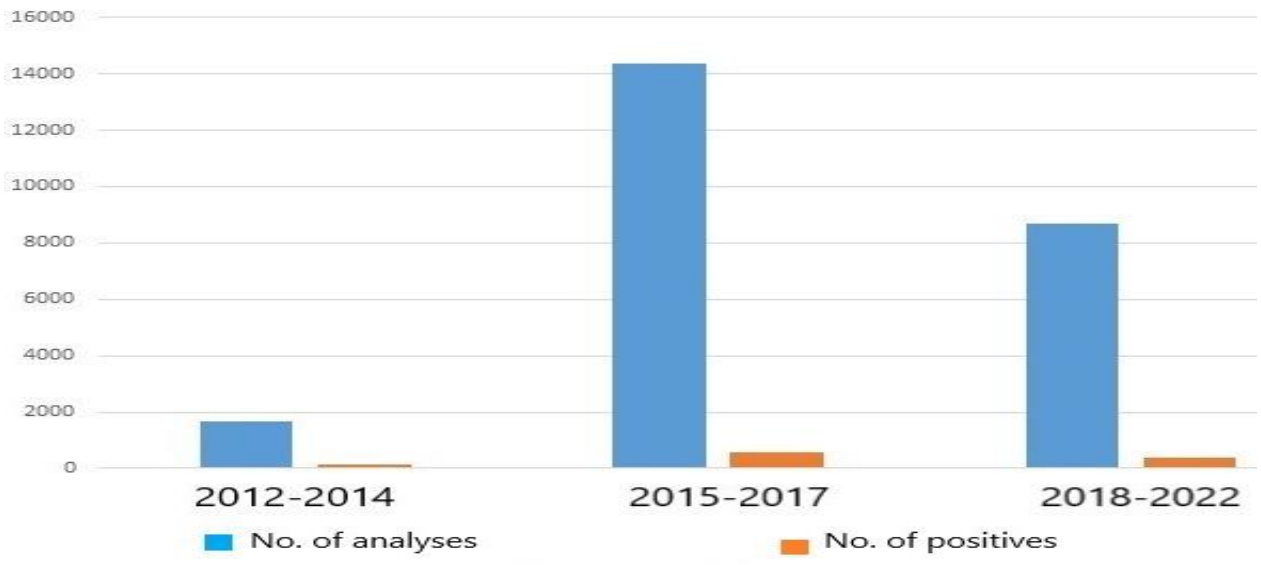

Figure 2. Breakdown of the number of tests before and after the agreement.

2018 to 2022 (post agreement period).

By gender

According to the present study, the proportion of HCV positivity was not related to gender in subjects under 15 years of age ($p > 5\%$) (Figure 3A). On the other hand, this proportion was related to sex in subjects aged between 15 and 50 ($p < 5\%$) (Figure 3B) and in subjects aged over 50 ($p < 5\%$) (Figure 3C). Whatever the age group, the proportion of HCV positivity is higher in men than in women (Figure 4).

Recipes for serological tests

In terms of recipes from serological tests, one test is invoiced at 7,000 f cfa. Before the agreement with ROCHE, the Institut Pasteur analyzed 1,655 tests, for a total revenue of 11,550,000 f cfa (23 10 USD). Although the cost of the test is lower than in the private sector, some patients are unable to pay for this serological screening analysis.

During the period of the agreement, the price of screening for viral hepatitis B and C was 2,000 f cfa for these three markers (AgHBS, total Ac Anti HBc, and Ac anti HVC), and the number of samples analyzed was 14,371, representing revenue of 28,742,000 f cfa (14,371 × 2000) for the IPCI, compared with 352,089,500 f cfa for the private sector.

After the agreement, the IPCI analyzed 8,661 samples for a revenue of 60,627,000 f cfa, compared with an estimated revenue of 212,194,500 f cfa for private-sector laboratories, where the unit cost is 24,500 f cfa.

DISCUSSION

In this study, a total of 24,687 samples were included over a period of 11 years, and hepatitis C was thought to be underdiagnosed due to the cost of testing long before Roche's agreement with the state of Côte d'Ivoire. The finding is that although the cost of testing has been reduced with Roche's agreement with the state of Côte d'Ivoire, and the number of samples diagnosed has increased from 2015 onwards, the proportions of virus positivity have been virtually the same over the years. This leads us to say that the HCV is endemic in our country.

The proportion of HCV infection positivity obtained in the present study virtually mirrored the same result obtained in a study conducted among blood donors in Côte d'Ivoire between 1997 and 2012, which was 4.4% (Enel et al., 2015). This constancy in the positivity rate could be explained by the fact that the CNTS data just like the IPCI data reflect the general population of Côte d'Ivoire on one hand and the maintenance of the status quo despite the presence of a national viral hepatitis control program in Côte d'Ivoire.

According to the current study, the proportion of HCV positivity was not related to sex in the under-15s ($p > 5\%$), which could be explained by the fact that people in this age group are not sexually active. In our study, the male sex was more concerned by viral hepatitis C infection, with a proportion of 5.26%, in contrast to the study carried out in Ghana in 2019, where positivity rather concerned the female sex, with a proportion of positivity

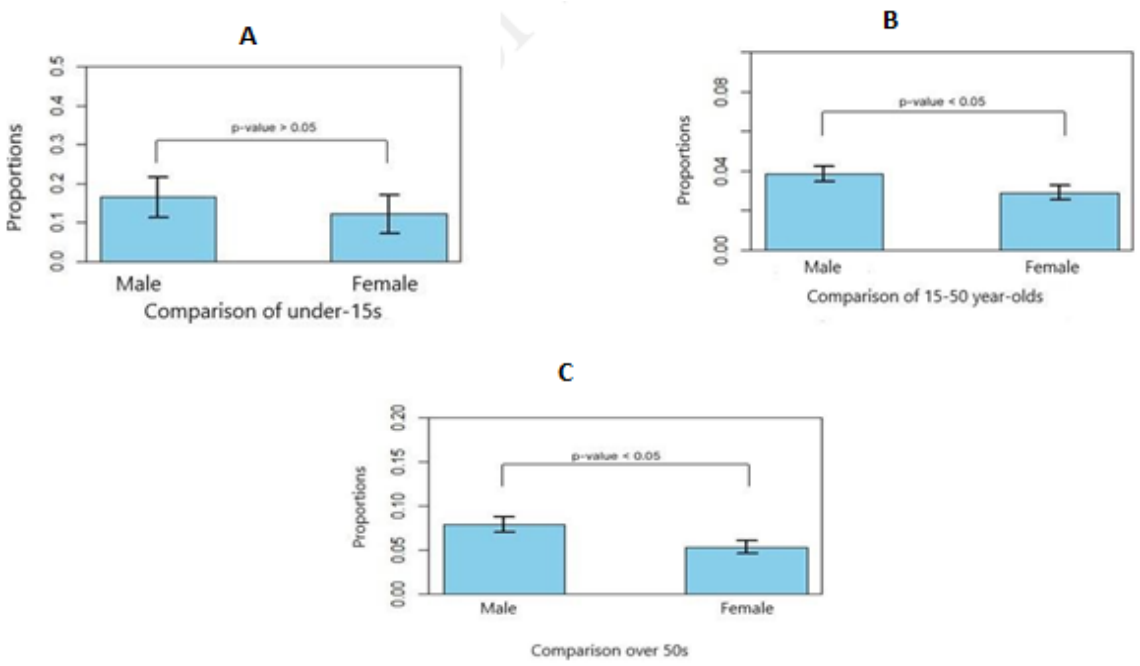

Figure 3. A, B, C: Comparison of the proportion of positivity by age.

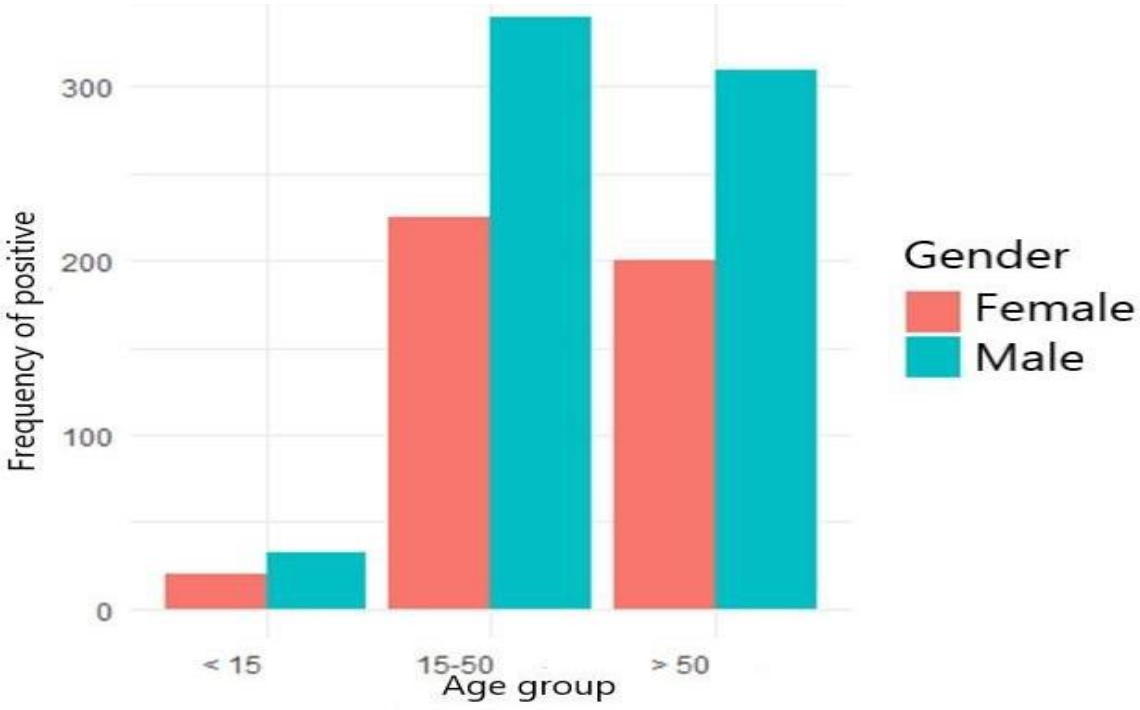

Figure 4. Proportions by gender and age group.

of up to 60% female in the study population (Masood and Mahbobeh, 2020). This large difference can be explained by the different objectives of the two studies. The Ghana study focused on at-risk subjects, while the present study did not. The age range in our study [1-87 years] was practically the same as in the study by

Kpossou et al. (2021) in Benin, with an age range of 0 to 86 years. This could be explained by certain age-related characteristics specific to West African countries. Our study showed that the proportion of HCV positivity was linked to gender in subjects aged over 15, as in the study conducted in Rwanda (Umutesi et al., 2017).

Conclusion

This study has enabled us to evaluate HCV screening at the Institut Pasteur de Côte d'Ivoire attended by the entire Ivorian population from 2012 to 2022. It also shows us the endemic nature of the HCV in Côte d'Ivoire. In our study, the male sex was more affected by viral hepatitis C infection, with a proportion of positivity of 5.26% versus 3.81% for the female sex. Despite financial support to facilitate screening, the seroconversion rate of the population did not show an increase in the number of subjects having contracted the HCV. This virus circulates in Côte d'Ivoire, but seems to cause less viral hepatitis than the hepatitis B virus. Systematic screening for this virus should nevertheless be pursued.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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Dynamics of the vaginal microbiome during the menstrual cycle of HIV positive and negative women in a sub-urban population of Kenya

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Vaginal microbiome (VM) is dominated by Lactobacillus for maintenance of vaginal health. The objective of this study was to characterize changes in the VM during the menstrual cycle of HIV positive (HIV+) and HIV negative (HIV-) women in a sub-urban population of Kenya. In this longitudinal study of 38 women-20 HIV+,18 HIV-high vaginal swabs were for genomic DNA and Gram stain and quantitative PCR (qPCR). qPCR nested on Gram stain showed high concentration of *L. iners* in normal VM, increasing during bacterial vaginosis (BV) and high levels of *L. jensenii* in women with BV while *L. crispatus* was absent. *G. vaginalis* increased from normal to BV. *A. vaginae* was absent in normal but detectable in intermediate gram stain and increased during BV. Gram stain showed BV was absent in HIV-ve women using condoms. Both groups had high concentration *L. iners* and *G. vaginalis*, harboured *A. vaginae*. Frequency and concentration of *L. crispatus* were less in HIV+ women, *L. jensenii* undetectable but condom use significantly higher. Menstrual cycle showed high concentration of *L. iners* and *G. vaginalis*. *L. crispatus* increased while *A. vaginae* decreased. At the initial phase, *L. jensenii* was low and undetectable thereafter. In this Kenyan population L. iners predominates normal VM, increased during BV. Both groups had high concentration of *L. iners* and *G. vaginalis*. Concentration of *L. crispatus* increased while *A. vaginae* decreased. Condoms and *L. crispatus* show protection against BV while *L. jensenii* does not. Both the presence and quantities of *L. crispatus* determine healthy VM.

Key words: Vaginal microbiome, HIV, qPCR, Lactobacilli, menstrual cycle.

INTRODUCTION

The vaginal micro-environment is dynamic and undergoes changes during the menstrual cycle in women of reproductive age. These changes correlate with the accompanying hormonal changes (Owen, 1975; Hay,

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2005; Farage et al., 2009). Studies show that changes occur in the vaginal microbiome during the normal menstrual cycle. However, most studies have been carried out in populations outside Africa such as in the United Kingdom (Wilks and Tabaqchali, 1987; Keane et al., 1997), Japan (Fujisawa et al., 1992) and in the United States of America (Onderdonk et al., 1986; Schwebke et al., 1999; Eschenbach et al., 2000; Ness et al., 2006). These studies found the most profound changes in vaginal microbiome to occur during menses. Only one study of African women has related the changes occurring in the vaginal microbiome with cyclic menstrual changes (Morison et al., 2005).

The recurrent imbalance that occurs in the equilibrium of vaginal microbiome favors the overgrowth of bacterial vaginosis (BV)-associated bacteria (Nyirjesy, 2008). The relative depletion of the resident Lactobacilli has been shown to be most profound at the time of menses, indicating that the vaginal microbiome become less stable during this event (Bartlett et al., 1977; Sautter and Brown, 1980; Onderdonk et al., 1986; Keane et al., 1997; Hay et al., 1997; Schwebke et al., 1997, 1999; Eschenbach et al., 2000; Wilson et al., 2007). This phenomenon has generally been attributed to the premenstrual decline in circulating levels of estrogen (Bradshaw et al., 2006; Wilson et al., 2007), which in turn affects the colonisation strength of the vaginal Lactobacilli, for example by limiting their capacity for epithelial adherence. The vaginal *Lactobacillus* microbiome are probably further challenged by the menstrual flooding of the vagina (Wilson et al., 2007), which is accompanied by a sharply rising pH, further exacerbating the unfavorable conditions for epithelial adherence of the Lactobacilli. Under such conditions the colonization resistance offered to BV-associated anaerobes can easily be overcome (Hay, 2005). Menses have also been shown to induce a wash-out of Lactobacilli. These mechanisms might explain why BV is less prevalent during pregnancy (Hay et al., 1994; Riggs et al., 2007; Song et al., 2020) and in postmenopausal women (Hillier and Lau, 1997), despite the relative estrogen deficiency especially in the latter group. To further demonstrate the important role that hormones might play in maintaining the balance in the vaginal ecosystem, one longitudinal study found women using hormonal contraceptives to have less prevalence due to greater remission of BV (Riggs et al., 2007).

Menses therefore represent a presumably critical challenge to the maintenance of the normal, Lactobacilli-dominated microbiome during the index cycle. This was clearly demonstrated in a previous study (Keane et al., 1997) where daily Gram-stained vaginal smears were prepared over the whole duration of a menstrual cycle among 21 volunteers. Seven of them who initially presented with basically normal vaginal microbiome showed BV-like overgrowth on further study follow-up, which was in all the cases preceded by a decrease of Lactobacilli within the first few days of the menstrual cycle. It is conceivable that following the decline in estrogenization of the vaginal epithelium, and the subsequent vaginal flooding with menstrual blood, Lactobacilli must replenish the vagina in due time within the first few days of the menstrual cycle. This study postulates that it is this re-colonization effort that determines the balance for vaginal microbiome stability during the rest of the cycle. Following disruption of the pH, it takes a normal population of Lactobacilli several hours to re-establish the known acidity (Boskey et al., 1999). In a similar subsequent study of 74 women, the 50 who had BV showed the overall rate of recovery of heavy growth of *Lactobacillus* to increase over the menstrual cycle. However, their results showed that this was not the case for the hydrogen peroxide (H_2O_2)-producing Lactobacilli (Eschenbach et al., 2000), suggesting that the acquisition of these species during the early follicular phase may determine the vaginal microbiome status during the entire cycle. Failure to recover a stable *Lactobacillus* microbiome apparently sets the scene for overgrowth of BV-associated anaerobic species during the index cycle as suggested in the study by Keane et al. (1997). In their observations, the development of BV was consistently preceded by decreased numbers of Lactobacilli during menses. In addition to these inescapable physiologic events, behavioural factors during menses may further compromise the delicate balance between the H_2O_2-producing Lactobacilli and BV-associated microorganisms. Douching after menses for instance has been found to act as a strong predictor of subsequent development of BV (Schwebke et al., 2004). This may be explained by the detrimental effect that douching has on the H_2O_2-producing Lactobacilli (Ness et al., 2002; Beigi et al., 2005), in addition to the antagonizing effect of menses (Brotman et al., 2008). A similar observation has been made for sexual intercourse during menses (Ness et al., 2004).

Beyond the critical menstrual period, the vaginal microbiome may continue to display transient shifts away from a Lactobacilli dominance (Priestley et al., 1997; Schwebke et al., 1999). Such episodes could represent a window phase of heightened vulnerability to the development of a true episode of BV, such as is induced by douching or sexual contact. A recent study showed that during menses the diversity of vaginal microbiome increased while on the contrary the concentration of *Lactobacillus* species decreased. The use of hormonal contraceptives in turn increased the Lactobacilli (Song et al., 2020). Of note in this respect is the observation in the Keane et al. (1997) study that, two of seven women converted to intermediate microbiome or BV, and had decreased Lactobacilli on the day prior to a change in the status as shown by Gram stain (Keane et al., 1997). The implications of these observations are critical to the understanding of the pathogenesis of BV as it may well be that established risk factors do not necessarily disrupt

a healthy vaginal microbiome, but merely superimpose on an already imbalanced microbiome. In one outstanding, example from a large prospective cohort study on vaginal douching, Hutchinson et al. (2007) concluded that contrary to consensus, douching may in fact sufficiently disrupt an already imbalanced flora to create BV, but not induce *de novo* BV or the acquisition of BV vaginosis-associated micro-organism. Similarly, Vallor et al. (2001) found sexual intercourse to be a risk factor for BV, an indication that coitus only poses a threat to the *Lactobacillus* if they are not granted sufficient time to recover following transient instability. In the latter study, antibiotic use and frequency of intercourse were shown to be factors that make it more difficult for H_2O_2-producing lactobacilli to recolonize the vagina. Recolonization also depends on the *Lactobacillus* spp. present, *Lactobacillus crispatus* and *Lactobacillus jensenii* showing more colonization resistance than *Lactobacillus gasseri* (Vallor et al., 2001). The latter study demonstrated the transient nature of *Lactobacillus* colonization. Within the study duration of 8 months, two thirds of the 101 women studied either lost or acquired colonization by *Lactobacillus*. Leppäluoto (2011) observed that temporary imbalance of the vaginal microbiome towards a BV-like profile following intercourse could be attributed to the accompanying changes in pH.

It therefore appears that the delicate balance of whether *Lactobacillus* will recolonize the vagina after menses is determined by certain factors, the key one being ability of the *Lactobacillus* strain present to overcome the prevailing resistance. Resistance to recolonization may be due to prolonged times of elevated pH as may happen in frequent intercourse. Other factors may include the systemic effects of hormones that may play a role at the receptor level in the vaginal epithelium. Srinivasan et al. (2010) observed that during menses the population of *G. vaginalis* increased alongside that of *Lactobacillus iners* while quantities of *L. crispatus* and *L. jensenii* decreased simultaneously. This trend was reversed at the end of menses. Similar observations were made in an earlier study (Schwebke et al., 1997). The former authors also observed that *Gardnerella vaginalis* was present in women with and without BV, and that the increase during menses may be linked to availability of a substrate in the menses, namely iron. Indeed, *G. vaginalis* contains vaginolysin that can perforate erythrocytes to release iron and activate immune markers of the vaginal epithelium (Gelber et al., 2008), causing the inflammation observed in BV. Vaginolysin, found in another Gram-positive genus (Gelber et al., 2008), belongs to cholesterol-dependent cytolysins (CDCs) produced by organisms that colonize and cause disease at mucosal surfaces (Tweten, 2005). In most cases, toxin production has been shown to be essential for maintenance of colonization and pathogenesis of invasive disease. Further, *G. vaginalis* species have been shown to produce sialidase (Santiago et al., 2011) that could exacerbate the pathogenesis of BV. Systemic effects of hormones may then only contribute to ongoing inflammation at the vaginal epithelium.

The pathogenesis of BV may further be unravelled through the appraisal of our current knowledge on the vaginal microbiome in relation to the menstrual cycle, when instability is critical to whether or not BV develops. Transient changes in the vaginal microbiome occur predominantly in the first part of the menstrual cycle, where the quantities of lactobacilli are significantly reduced (Keane et al., 1997; Morison et al., 2005; Song et al., 2020). Vaginal colonization by Lactobacilli is believed to confer multiple benefits to women, among them being the inhibition of the development of BV, a condition associated with many undesirable effects such as preterm delivery (Hillier et al., 1995; Leitich et al., 2003; Wilks et al., 2004; Fettweis et al., 2019) and increased vulnerability to other STIs (Allsworth et al., 2008; Cherpes et al., 2003; Kaul et al., 2007; Doerflinger et al., 2014; Bayigga et al, 2019), including HIV infection (Cohen et al., 1995; Sewankambo et al., 1997; Taha et al., 1998; Martin et al., 1999). Studies show that vaginal microbiome dominated by non-*Lactobacillus* bacteria increases the risk of HIV infection (Wang et al., 2023). Further it has been shown that a combination of specific *Lactobacillus* predominance as well as its concentration may both be crucial for maintenance of the dynamic physiologic balance of healthy vaginal microbiome (De Seta et al., 2019). The purpose of the current study was to describe by Gram stain and quantitative PCR, the dynamics of the vaginal microbiome of HIV^+ and HIV^- premenopausal women during the menstrual cycle in a sub-urban population of Kenya.

METHODS

Study population

The characteristics of the study population are described in detail elsewhere (Kiama et al., 2014) and summarized here in Table 1. The study was approved by the ethical review board of Kenyatta National Hospital/University of Nairobi (Registration No. P122/8/2005).

Laboratory screening tests

The study participants were screened for HIV-1 infection, pregnancy, and syphilis. Two high vaginal swabs were taken for *Trichomonas vaginalis* culture and for the diagnosis of candidiasis as assessed by microscopic examination for the presence of budding yeasts or pseudohyphae in a drop of 10% KOH. One endocervical swab was used for the combined *Clamydia trachomatis* and *Neisseria gonorrhoeae* PCR. These screening tests were carried out as described previously (Kiama et al., 2014). The $CD4^+$ T cell count was performed by flow cytometry on Becton Dickinson FACS automatic Count System with fluorochrome-labeled antibodies and a fluorescent beads standard for enumeration. Stained samples were analysed on a Facs-Calibur

Table 1. Population characteristics of participants at enrolment into the study.

Parameter	HIV$^+$ (n=20)	HIV$^-$ (n=18)	P value
Age (years)			
21-28	4 (20.0)	4 (22.2)	0.78
29-36	10 (50.0)	7 (38.9)	
37-44	6 (30.0)	7 (38.9)	
CD4 count (cells/µL)			
250-500	4 (20.0)	1 (5.6)	0.001
501-750	11 (55.0)	1 (5.6)	
751-1000	3 (15.0%	8 (44.4)	
1001-1500	2 (10.0)	8 (44.4)	
Gram stain			
Grade 0	0 (0.0)	1 (5.6)	
Grade I	14 (70.0)	14 (77.8)	NA
Grade II	0 (0.0)	0 (0.0)	
Grade III	5 (25.0)	2 (11.1)	
No score	1 (5.0)	1 (5.6)	
Antibiotic prophylaxis			
Yes	13 (65.0)	0 (0.0)	<0.001
No	7 (35.0)	18 (100.0)	
Marital status			
Married	15 (75.0)	9 (50.0)	0.01
Single/separated	2 (10.0)	9 (50.0)	
Widow	3 (15.0)	0 (0.0)	
Condom use			
Yes	12 (60.0)	4 (22.2)	0.02
No	8 (40.0)	14 (77.8)	
Level of schooling			
Primary and below	9 (45.0)	4 (22.2)	0.33
Secondary	10 (50.0)	13 (72.2)	
Tertiary	1 (5.0)	1 (5.6)	

machine using CELLQuest Software (Becton Dickinson).

Sample collection and grading during follow-up

Specimens were collected three times per cycle for two consecutive menstrual cycles as follows: Follicular phase (Day 5-8); Ovulaton phase (Day 12-15); Luteal phase (Day 19-22) to allow for flexibility of scheduled visits.

For the 38 women (20 HIV$^+$ and 18 HIV$^-$) that were followed up, two high vaginal swabs were obtained at subsequent visits as follows: with a non-lubricated speculum in place, sterile cotton swabs were consecutively inserted into the vaginal vault. Each swab was rotated against the lateral vaginal wall at the mid-portion of the vault and carefully removed to prevent contamination with the vulva and introitus microbiome. The first swab was used to make a Gram stain. The second swab was transported to the laboratory in a dry sterile tube for DNA extraction. Gram stain specimens were analysed for the composition of the vaginal microbiome by microscopy according to the Nugent criteria (Nugent et al., 1991). An additional category of the Gram stains known as grade 0 was included to represent the smears lacking bacteria cells (Verhelst et al., 2005).

Statistical analysis

Prevalence rates were compared between groups through Chi-square test or Fischer's Exact Test. Statistical significance was accepted at the significance level α=0.05. All analyses were

Table 2. Distribution of Nugent scored vaginal microbiome grades among HIV+ve and HIV-ve women during different phases of two menstrual cycles. Percentages in parentheses.

Grade	HIV +ve (n = 41)			HIV -ve (n = 33)		
	Follicular	Ovulation	Luteal	Follicular	Ovulation	Luteal
0	2 (2.4)	4 (4.9)	0 (0.0)	1 (1.5)	1 (1.5)	0 (0.0)
I	42 (51.2)	49 (59.8)	53 (64.6)	41 (62.1)	45 (68.2)	44 (66.7)
II	18 (22.0)	12 (14.6)	15 (18.3)	11 (16.7)	8 (12.1)	9 (13.6)
III	17 (20.7)	16 (19.5)	14 (17.1)	11 (16.7)	12 (18.2)	11 (16.7)
Missing	3 (3.7)	1 (1.2)	0 (0.0)	2 (3.0)	0 (0.0)	2 (3.0)
Total	82	82	82	66	66	66

performed with statistical software package PASW v18.0 (Chicago, IL).

DNA extraction from dry vaginal swabs

The dry swab specimen from each patient was swirled for 15 s in 1200 µl of lysis buffer (20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton). Aliquots of 200 µl were prepared. To each aliquot was added 5 µl 10% SDS to final concentration of 0.25%. Fifty units of mutanolysin (25 U/µl) (Sigma, Bornem, Belgium) were added and the samples were incubated for 15 min at 37°C. After the addition of 20 µl Proteinase K (25 mg/ml) the samples were incubated for 15 min at 55°C and vortexed every 5 min. Afterwards, 1800 µl Nuclisens Easymag buffer (BioMérieux, La Balme-les-Grottes, France) was added and the samples were incubated for 10 min at room temperature. Subsequently, 2.0 ml of the processed sample was added to Easymag disposable caps. A 100 µl mixture of magnetic silica and Easymag extraction buffer 3 added. DNA extraction from the pretreated swab medium was then performed on the NucliSENS EasyMAG system according to the manufacturer's instructions. The DNA extracts were stored at -80°C for later use.

Real-time PCR (qPCR)

The qPCR Core Kit for SYBR Green I (Eurogentec, Luik, Belgium) was used and analysis performed on the ABI 7300 real-time PCR system (Applied Biosystems, Foster City, CA).

Reactions were done in PCR mixtures containing 2.5 µl of DNA extract, 2.5 µl of 10x Reaction Buffer, 3.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 0.625 U HotGoldStar *Taq* polymerase, 0.75 µl SYBR® Green I, diluted 10-fold in DMSO and the appropriate primer concentration. Core Kit qPCR primers were used. The reaction mixture was adjusted to 25 µl with HPLC grade water. Each run included a standard series, and each sample was run in triplicate. In case the result was not in the range of the standard curve, the samples were diluted tenfold and re-analyzed in triplicate. The median $\log_{10}$ cells/ml was expressed as per 1 ml elution buffer.

Statistical analysis

Data were analyzed under the non-parametric assumption, taking into consideration the $\log_{10}$ [count] distributions of species under study did not approximate the normal distribution. For any given category (grades I-IV), the distribution of concentrations ($\log_{10}$ cells/ml) of each species was expressed as the median count and the accompanying interquartile range (IQR). Between-group comparisons of distributions were performed with the Mann-Whitney U-test for two groups and with the Kruskal Wallis test for multiple groups. Correlations between the different species were determined by the Spearman (rank) test and reported as Spearman's rho value (r). All analyses were performed using SPSS v15 software (Chicago, Illinois).

RESULTS

Cohort characteristics

The baseline demographic characteristics of the 20 HIV^+ and 18 HIV^- women are presented in Table 1. All the participants were sufficiently literate to follow the appointment schedule. For most parameters measured at enrolment, no significant differences existed between the two groups. The HIV^+ women had significantly lower CD4+ T cell counts (p=0.001). Another difference in the groups directly related to the HIV-status was antibiotic prophylaxis intake among the HIV^+ group (p<0.001). The Kenya national guidelines for treatment and care for HIV persons allow continuous antibiotic intake for prevention of malaria and recurrent bacterial infections. Furthermore, 75% of the HIV^+ participants were married compared to 50% HIV^- ones (p=0.01).

The Gram stain and culture results for this population were previously analyzed in Kiama et al. (2014).

Nugent score

Table 2 shows the number of grades I, II and III Gram stains was similar throughout the phases of the menstrual cycle in both study groups.

Fluctuation of the Gram stain score

Table 3 shows the fluctuation in Gram stain score over two menstrual cycles. While BV was virtually absent in HIV-ve women using condoms, half of the HIV+ve women using condoms had a disturbed microbiome on three or more visits. Of the women not using condoms, majority had one or more episodes of BV during the scheduled visits.

Table 3. Percentages of the fluctuation of the Gram stain scores of HIV -ve and HIV +ve women taken at six visits each, spanning two menstrual cycles.

Total Number	HIV -ve		HIV +ve	
	Condom Use			
	No (27)	Yes (6)	No (17)	Yes (24)
Invariably normal	33.3	83.3	47.1	20.8
Single intermediate or BV episode	11.1	16.7	11.8	29.2
Two intermediate or BV episodes	14.8	0	5.8	0
Three or more intermediate or BV episodes	29.6	0	17.6	37.5
Invariably intermediate or BV episode	11.1	0	17.6	12.5

Real-time PCR

Figure 1 shows the results of real-time PCR analysis of different bacterial species based on Gram stain category. There was a high load of *L. iners* in the normal vaginal microbiome, which increased during BV. There were high levels of *L. jensenii* in half of the women with BV, while *L. crispatus* was absent in BV cases. *G. vaginalis* concentration increased progressively from normal to BV microbiome while *Atopobium vaginae* was absent in normal microbiome, but was detectable in intermediate Gram stain and increased progressively during the BV phase.

Figure 2 shows the results of real-time PCR analysis based on HIV status. There were high concentrations of *L. iners* and *G. vaginalis* in both the HIV^+ and HIV^- groups. The frequency and concentration of the protective *L. crispatus* were less in the HIV^+ group of women. *A. vaginae* was present in both groups, but *L. jensenii* was not detectable in the HIV^+ women.

Figure 3 shows the real-time PCR analysis based on phase of menstrual cycle. There was high concentration of *L. iners* and *G. vaginalis* throughout the menstrual cycle. The concentration of *L. crispatus* increased, while the concentration of *A. vaginae* decreased across different phases of the menstrual cycle. *L. jensenii* was not detectable after the initial phase of the menstrual cycle, where its concentration was comparatively low.

DISCUSSION

This is the first study to use a combination of Gram stain and real-time PCR to investigate the vaginal microbiome of African HIV^+ and HIV^- women. Vaginal colonization by Lactobacilli is believed to confer multiple benefits to women, among them being the inhibition of the development of BV, a condition associated with many undesirable effects such as preterm delivery (Hillier et al., 1995; Leitich et al., 2003; Wilks et al., 2004; Fettweis et al., 2019) and increased vulnerability to other STIs (Allsworth et al., 2008; Cherpes et al., 2003; Kaul et al., 2007; Doerflinger et al., 2014; Bayigga et al, 2019), including HIV infection (Cohen et al., 1995; Sewankambo et al., 1997; Taha et al., 1998; Martin et al., 1999). Thus, molecular characterization of the Lactobacilli and other bacteria species that colonize the vaginas of HIV^+ and HIV^- women has a high potential to help in understanding the dynamism of microbiome in health and disease. Both the specific *Lactobacillus* predominance as well as its quantities are crucial for maintenance of the dynamic physiologic balance of healthy vaginal microbiome (De Seta et al., 2019). We found interesting patterns of dynamism of the vaginal microbiome to occur during the menstrual cycle by using real-time PCR to quantify *L. crispatus*, *L. jensenii*, *L. iners*, *G. vaginalis* and *A. vaginae*.

Gram stain and condom use

BV was found to be absent in all HIV-ve women using condoms. This situation was replicated in about 50% of HIV+ve women who also used condoms. Not using condoms appears to predispose to the acquisition of BV. A previous study (Hutchinson et al., 2007) also found the use of condoms to be associated with decreased risk for acquisition of BV as well as the spread BV-associated microflora. It has previously been postulated that the presence of BV increases the risk for HIV acquisition (Wang et al., 2023). It may thus be that the HIV+ve women in this study started using condoms when they already had BV. The presence of BV at the initiation of the study has previously shown to have no impact on incidence (Yotebieng et al., 2009). Recurrence may however be prevented. Condoms can offer protection against BV as well as other sexually-transmitted infections (Fethers et al., 2008).

Real-time PCR analysis based on Gram stain category

Application of real-time PCR to quantify vaginal microbiome has previously been used by other groups. Molecular quantification eliminates culture bias and

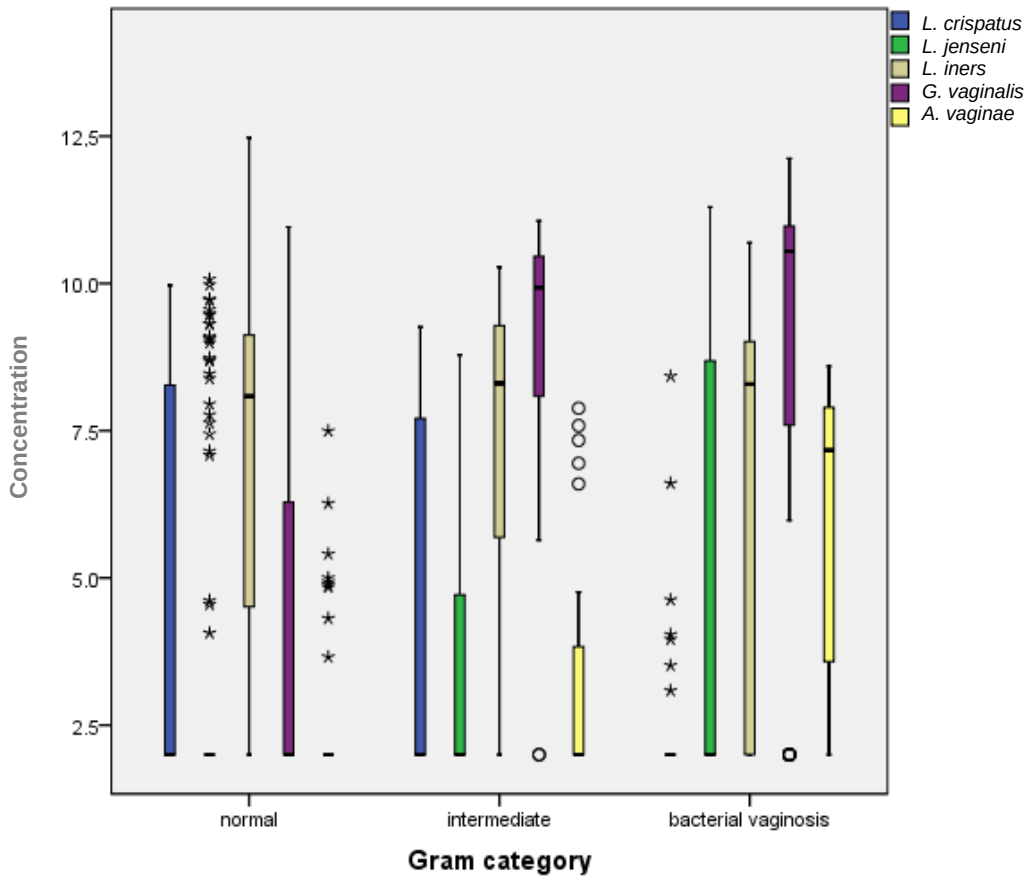

Figure 1. Real-time PCR analysis based on Gram stain category.

provides for objective interpretation of results and also gives more detailed information of resident species irrespective of density, and can lead to the recognition of novel species (Rodriguez-Jovita et al., 1999; Verhelst et al., 2004). It also allows detection of biologically inactive biofilm communities (Swidsinski et al., 2005) that are inevitably missed out by culture methods. In the vagina of healthy premenopausal women, Lactobacilli are the predominant bacteria (Swidsinski et al., 2005; Zhou et al., 2009). However, in this study, we found a high load of *L. iners* to be equally represented in normal, intermediate and BV microbiome. Further, De Backer et al. (2007) found *L. iners* to be increasingly associated with disturbed vaginal microbiome unlike previously held presumption that Lactobacilli are only associated with normal flora. *L. iners* was also present in normal and intermediate Gram stains, which is in agreement with Anukam et al. (2006) who found *L. iners* to be the predominant Lactobacilli in healthy premenopausal Nigerian women (64% of 241). Other studies have also reported *L. iners* to be one of the predominant lactobacilli in vaginal microbiome of BV-free Caucasian women in Sweden (Vasquez et al., 2002) and Canadian women (42% of 19) without symptoms or signs of urinary tract infection (Burton et al., 2003). Although previous studies omitted *L. iners* due to culture bias (De Backer et al., 2007), culture-independent studies have recently shown that *L. iners* is one of the predominant *Lactobacillus* in vaginal microbiome (Vasquez et al., 2002; Fredricks et al., 2005; Zhou et al., 2007). Here, we show that *L. iners* is the predominant *Lactobacillus* in these HIV^+ and HIV^- Kenyan women, irrespective of the Nugent grading of their vaginal microbiome. It was concluded that *L. iners* is part of biologically inactive biofilm community in the vaginal Microbiome.

Also in agreement with De Backer et al. (2007) was the finding that *L. jensenii* was present sporadically in all the Nugent grades. There were high levels of *L. jensenii* in half of the women with BV and very low levels in the women with normal vaginal microbiome. Our results are also in agreement with Anukam et al. (2006) who detected *L. jensenii* only in a few women with normal Nugent scores from their Nigerian study population, using PCR-denaturing gradient gel electrophoresis (DGGE).

In this study, we found *L. crispatus* to be either absent or in very low concentration in women with BV, which is consistent with the known protective role of this species in normal vaginal microbiome (Verstraelen et al., 2009).

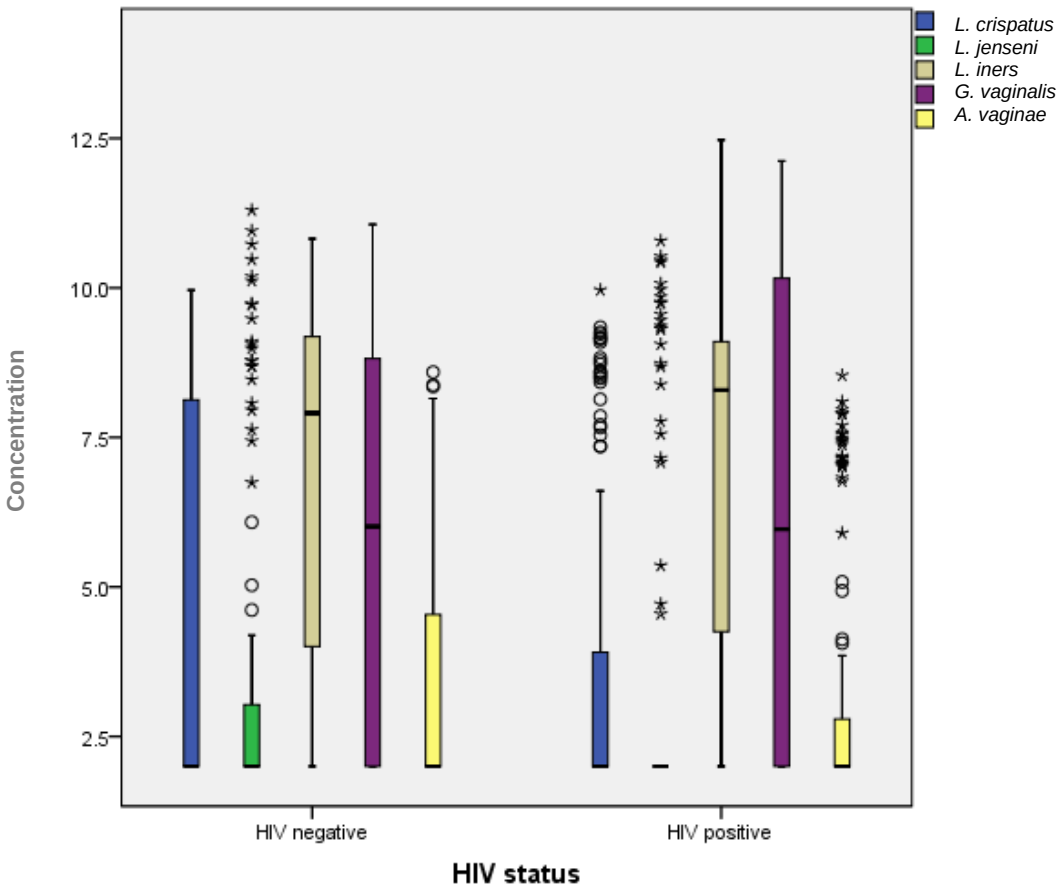

Figure 2. Real-time PCR analysis based on HIV status.

L. crispatus has also been found to offer vaginal colonization resistance against Group B Streptococci during pregnancy (Starc et al., 2022). Although De Backer et al. (2007) found *L. crispatus* to be present in varying concentrations across all Nugent grades, they used a cohort inclusive of pregnant Belgian women. However, the present study specimen collection was based on the menstrual cycle, and most BV cases coincided with the early phase, as was previously shown (Morison et al., 2005). It has also been shown that infrequent specimen collection can lead to underestimation of BV or to omission of the dynamism of vaginal microbiome (Brotman et al., 2010).

L. crispatus and *L. jensenii* showed an interesting inverse relationship where *L. crispatus* decreases from the normal microbiome to almost being undetectable in BV; *L. jensenii* is almost undetectable in normal microbiome but increases progressively to peak at BV microbiome. This study provides evidence to contradict the currently held hypothesis that both *L. crispatus* and *L. jensenii* must be present in normal vaginal microbiome. Vasquez et al. (2002) also found *L. crispatus* to be the single predominant *Lactobacillus* in the vagina. The findings of the present study correspond with those of Hawes et al. (1996) who found that lack of vaginal H_2O_2-producing lactobacilli or presence of only non-H_2O_2-producing lactobacilli were risk factors for acquisition of BV. It appears that *L. jensenii* alone, though known to be a H_2O_2-producer (Hawes et al., 1996; Antonio et al., 1999), is not protective since it was very low in normal microbiome and high in BV. *L. jensenii* has previously been shown to exhibit both poorer colonization strength and poorer colonization resistance, in comparison to *L. crispatus* (Verstraelen et al., 2009). We confirm that it is *L. crispatus* that apparently tips the balance in these women. We propose that in these women only *L. crispatus* is critical in vaginal health, and that possibly, the depletion of this *Lactobacillus* contributes to development of BV. Fettweis et al. (2019) found low concentrations of *L. crispatus* in the vagina to be implicated in preterm delivery. It has previously been suggested that existing differences in microbial communities of Caucasian and African women may well account for discrepancies in their susceptibility to BV and other vagina infections (Royce et al., 1999; Zhou et al., 2007). It is notable that all grades of vaginal microbiome

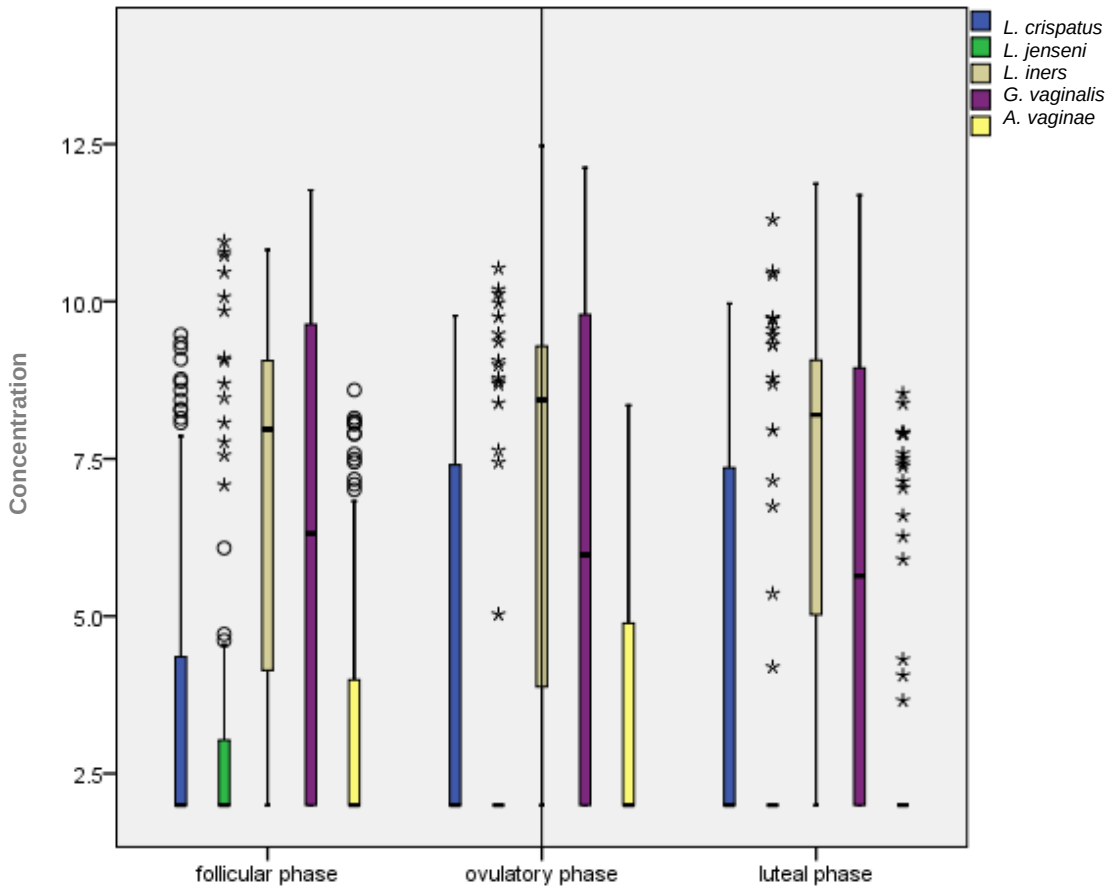

Figure 3. Real-time PCR analysis based on phase of menstrual cycle.

had at least two species of *Lactobacillus*, which is in conformity with the proposal that the function of lactic acid production in vaginal microbiome is highly conserved (Zhou et al., 2007; Ravel et al., 2010), which underscores its importance.

In this study, the concentration of *G. vaginalis* increased progressively to reach a peak in BV-associated microbiome. This finding is in agreement with that of De Backer et al. (2007) whom, also using real-time PCR, found *G. vaginalis* to be present in all grades of the vaginal microbiome. Burton et al. (2003) using DGGE and sequence analysis found that the presence of *G. vaginalis* did not necessarily exclude *Lactobacilli* from the vaginal ecosystem; this contradicts an earlier hypothesis that *G. vaginalis* only occurred where *Lactobacilli* were depleted (Martin et al., 1999; Baeten et al., 2009). We found a strong positive correlation of occurrence between *G. vaginalis* and *L. iners,* irrespective of the Nugent score. It was proposed that these two species possibly contribute to the comparatively higher pH found in women of African descent (Royce et al., 1999; Ravel et al., 2010), which may play a role in the high prevalence of BV in this population. Ravel et al. (2010) found that vaginal bacterial communities not dominated by *L. crispatus* tend to have slightly higher pH values. The vaginal communities in this study population were dominated by *L. iners* irrespective of Nugent grade, which may imply that these women naturally have higher vaginal pH values. This implies that they tend to have a reduced colonization resistance, a factor that could easily predispose to other less resilient species, leading to development of BV. The differences in the *Lactobacillus* communities present in African women as shown in this study and others (Anukam et al., 2006; Ravel et al., 2010) compared to Caucasian women (Eschenbach et al., 1989; Hawes et al., 1996), coupled with sexual habits and practices may together influence the high susceptibility of African women to BV (Bukusi et al., 2006; Allsworth and Peipert, 2007; Brotman et al., 2008). It has been reported that *L. crispatus*, even when accompanied by the other *Lactobacillus* species, offers significant stability to vaginal microbiome in contrast to *L. iners* dominated microbiome (Verstraelen et al., 2009).

The concentration of *A. vaginae* was found to be either very low or absent in normal microbiome, detectable in intermediate Gram stain and increasing progressively

during the BV phase. Other recent studies have documented the presence of *A. vaginae* in vaginal microbiome. Since its discovery (Jovita et al., 1999) and subsequent association with BV (Verhelst et al., 2004; Fredricks et al., 2005), some researchers have proposed that *A. vaginae* can reliably be used to indicate diagnosis for BV (Feris et al., 2004; Burton et al., 2005; De Backer et al., 2007). These suggestions were based on the observation that *A. vaginae* was present in a high percentage of BV patients. Burton et al. (2005) detected *A. vaginae* in 50% of Canadian BV patients and Ferris et al. (2004) found that *A. vaginae*-specific PCR assays were negative in all women with normal vaginal Gram stains (35 women in total). They suggested that *A. vaginae* is rarely if ever a component of normal vaginal flora. De Backer et al. (2007) found high concentrations of *A. vaginae* in grade III samples. In this study, we found in addition that *A. vaginae* had an inverse relationship with *L. crispatus*. In BV microbiome where *A. vaginae* was the highest, *L. crispatus* was absent or not detectable, confirming these earlier reports. It is notable that in our results, the PCR analysis of the normal microbiome resembles that of the luteal phase, confirming earlier reports that vaginal microbiome is most unstable in the early phase of the menstrual cycle (Keane et al., 1997; Eschenbach et al., 2000; Morison et al., 2005). The very low presence of *A. vaginae* in normal microbiome and the subsequent resurgence in BV microbiome may be explained by Walker's hypothesis (Walker, 1992) of microbial community structures referred to as "drivers and passengers" theory. It postulates that for an ecosystem to function there are "driver" species (in our case *L. crispatus*) that strongly influence the community structure where they occur. Other species in the community constitute "passenger" species that have no major influence. Further, Ravel et al. (2010) postulated that undetectable members of a community (e.g. *A. vaginae* in normal Gram stain) may serve as a "seed bank" of species whose numbers multiply when favourable conditions arise, as would happen for example in this case when *L. crispatus* becomes depleted for one reason or another (in the BV-associated microbiome).

Real-time PCR analysis based on HIV status

So far only a few studies have compared the vaginal microbiome of HIV^+ and HIV^- women (Spear et al., 2008; Spear et al., 2011; Apalata et al., 2021). This is the first study to compare the vaginal microbiome of African HIV^+ and HIV^- women, sampled severally during the menstrual cycle, using culture-independent methods. All the HIV^- women had CD_4 counts above 500, except one with 488. 56.1% of the HIV^+ women had CD_4 counts of 250≥ while the rest had a count above 500, and none were on antiretroviral therapy. Analysis of PCR results based on HIV status showed a high concentration of *L. iners* and *G. vaginalis* in both the HIV^+ and HIV^- groups. Although condom use was significantly higher in HIV^+ women, the number of women harbouring the protective *L. crispatus* as well as the concentration of *L. crispatus* were remarkably lower compared to HIV^- women. *A. vaginae* was present in both groups, but *L. jensenii* was not detectable in the HIV^+ women. Spear et al. (2011) also found *L. jensenii* to be absent or present at relatively low levels in all except one out of 46 women studied. Since *L. jensenii* is known to have poor colonization resistance (Verstraelen et al., 2009), it is possible that increased biodiversity in HIV^+ women (Spear et al., 2008; Kiama et al., 2014) leads to the displacement of this species. We did not find that HIV infection influences the incidence of BV, which is in agreement with earlier reports (Greenblatt et al., 1999; Watts et al., 2006). However, other researchers (Apalata et al., 2021) found a higher incidence of BV in HIV^+ women compared HIV^- ones based on Nugent scoring. Since it has been shown that vaginal microbiome dominated by non- *Lactobacillus* bacteria increases the risk of HIV infection (Wang et al., 2023), it can be concluded that it is the absence of Lactobacillus that causes the higher incidence of BV in HIV^+ women. The observation that both groups of women in the current study harbored *L. crispatus* and *A. vaginae* may explain why we did not find differences in the prevalence of BV between them, since *A. vaginae* was suggested to be a reliable indicator for BV. It appears that *L. iners* and *G. vaginalis* are a fixed structure of the vaginal communities of these women irrespective of the prevailing circumstances. It is suggested that the short-term temporal dynamics of vaginal communities are determined by the species proportions of *L. crispatus* and *L. jensenii*, which in turn determine the relative resistance and resilience to the inevitable challenges of systemic homeodynamism, local disturbances as well as pertinent individual behaviors, habits, and practices. Given such an ecosystem, opportunistic and pathogenic species will more likely gain a foothold in communities that exhibit low stability (Hobbs and Huenneke, 1992; Verstraelen *et al*., 2009). This is a likely situation where *L. iners* predominates, since it is known to offer less colonization resistance due to low H_2O_2 production (Eschenbach et al., 1989; Hillier et al., 1993; Verstraelen et al., 2009), coupled with the observation that such communities tend to have slightly higher pH values (Ravel et al., 2010) and are less stable than those where *L. crispatus* predominates (Verstraelen et al., 2009).

Data obtained from HIV^+ pregnant indicate that a disturbed vaginal microbiome raises the risk for horizontal transmission of the virus (Frank et al., 2012). The current study however used non-pregnant reproductive-age women. It has been found that the presence of BV increases the risk for acquisition of HIV as well as other sexually transmitted infections because of the compromised integrity of the vaginal mucosal barrier (Doerflinger et al., 2014; Bayigga et al., 2019).

Real-time PCR analysis based on phase of menstrual cycle

Analysis based on the phase of the menstrual cycle, showed a high concentration of *L. iners* and *G. vaginalis* throughout the menstrual cycle. In all the three angles of analysis done here, these two species appear present in high concentrations. We propose that they are a fixed structure of the vaginal communities of these women irrespective of the prevailing circumstances. The unaltered presence of *L. iners* in normal vaginal microbiome of African women was also document by other researchers (Jespers et al., 2017). The concentration of *L. crispatus* increased while concentration of *A. vaginae* decreased throughout the menstrual cycle. This trend is exactly a reversal of what we observed in PCR analysis based on Gram stain scores. It has been previously reported that vaginal microbiome tends to be less stable during the early phase of the menstrual cycle (Keane et al., 1997; Eschenbach et al., 2000; Morison et al., 2005). This instability appears to be coupled to the decrease and subsequent displacement of *L. jensenii* and *A. vaginae* from follicular through to the ovulation phase. *L. jensenii* was not detectable after the initial phase of the menstrual cycle where it was comparatively low. Dynamic systemic changes in hormonal levels occurring during the cycle are usually accompanied by dramatic changes in the vaginal epithelium that influence the type and volume of secretions, the pH as well as the adherence capabilities of resident communities in the face of menstrual flooding (Owen, 1975; Brotman et al., 2008; Farage et al., 2009). During the follicular phase all the three lactobacilli studied were present together with *G. vaginalis* and *A. vaginae*. This state of vaginal microbiome is mirrored by the intermediate Gram stain category. It appears to be a transient stage that the microbiome goes through before the full effects of the systemic and local changes take their full effect. Immediately after, in the ovulatory phase, instability is seen in the displacement of *L. jensenii* and the accompanying steady concentration of *A. vaginae*. The luteal phase is driven by increased secretion of two groups of steroids, namely progesterones and estrogens (Owen, 1975; Farage et al., 2009). The qPCR Figure 3 of the vaginal microbiome during this phase resembles closely the one obtained from the normal microbiome where both *L. jensenii* and *A. vaginae* were either absent or undetectable. We provide evidence here that the instability witnessed in the early stage of the menstrual cycle is due to decreasing levels of *L. jensenii* as well as increasing levels of *A. vaginae*. Other researchers found this trend to be associated with increased concentrations of proinflammatory cytokines (Jespers et al., 2017).

Srinivasan et al. (2010) observed that during menses the population of *G. vaginalis* increased alongside that of *L. iners* while quantities of *L. crispatus* and *L. jensenii* decreased simultaneously. Similar observations were made in an earlier study (Schwebke et al., 1997). The former authors also observed that *G. vaginalis* was present in women with and without BV. *G. vaginalis* contains vaginolysin that can perforate erythrocytes to release iron and activate immune markers of the vaginal epithelium (Gelber et al., 2008), causing the inflammation observed in BV. Vaginolysin belongs to cholesterol-dependent cytolysins produced by organisms that colonize and cause disease at mucosal surfaces (Tweten, 2005). The implication is that in these women inflammation of the vaginal epithelium constantly poses the danger of development of BV. Toxin production has been shown to be essential for maintenance of colonization and pathogenesis of invasive disease (Tweten, 2005). Systemic effects of hormones may then only contribute to ongoing inflammation at the vaginal epithelium.

Conclusions

This study shows that *L. iners* is the predominant *Lactobacillus* in these HIV^+ and HIV^- Kenyan women, irrespective of the Nugent grading of their vaginal microbiome. This study provides evidence to contradict the currently held hypothesis that both *L. crispatus* and *L. jensenii* must be present in normal vaginal microbiome. We propose that only *L. crispatus* is critical in vaginal health, and that possibly, the depletion of this *Lactobacillus* contributes to development of BV. We suggest that *L. iners* and *G. vaginalis* are a fixed structure of the vaginal communities of these women irrespective of the prevailing circumstances. Condom use was found to be protective against BV.

The results showed that *A. vaginae* had an inverse relationship with *L. crispatus*. In BV-associated microbiome where *A. vaginae* was the highest, *L. crispatus* was absent or undetectable. On the other hand, the concentration of *L. crispatus* increased while the concentration of *A. vaginae* decreased throughout the menstrual cycle. We confirm that the instability observed in the vaginal microbiome during the early phase of the menstrual cycle is coupled to the decrease and subsequent displacement of *L. jensenii* and *A. vaginae* from follicular through to the ovulation phase.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Review

A comparative study of transgenic cotton development, impacts, challenges and prospects with respect to China and Africa

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Cotton is grown in about 101 countries with about 10 countries including China contributing highest quantity. Africa contributes less than 5% to the global demand for cotton. Processing of cotton generate so many businesses for the rural people of China and Africa. Like other cotton producing continents, the majorly cultivated species of cotton in China and Africa is *Gossypium hirsutisms* Gh (Upland Cotton) and is mostly grown by the smallholder farmers using crop rotation with few large plantations. China and Africa climate condition encourage pest growth which brings about pest attack on cotton followed by yield losses. Effort towards solving this problem was based on integration of transgenic cotton into cotton farming. It has been found that *Bacillus thuringiensis* strains produce crystal (Cry) and cytolytic (Cyt) toxins at the beginning of sporulation and during the stationary growth phase. These crystals are aggregate of proteins encoded by Cry genes and they have insecticidal properties. The Bt Cry genes have been isolated and used to transform cotton seed thus, the term Bt cotton/transgenic cotton which now have in built Cry gene to resist insect attack. In cotton, Bt gene is mostly expressed in the green parts of plant compared to the non-green parts and in the young plants compared to the older plants. This work therefore focused on cotton farming, the Bt crystal, Bt gene, methods of transformation of cotton with Bt gene, Bt gene expression level, resistances, mode of action, limitations and possible recommendations with respect to its use in China and Africa.

Key words: Cotton, cotton farming, genetic engineering, *Bacillus thuringiensis* (Bt), Bt cotton, Bt gene, Bt crystal, China, Africa.

INTRODUCTION

Cotton is grown in about 101 countries with about 10 countries contributing highest quantity. One of the majorly cotton producing nations on earth is China. Africa contributes less than 5% to the global demand for cotton (OECD-FAO Agricultural outlook, 2020). Cotton is an important rare economic story in sub Saharan Africa, a major source of foreign exchange earning in more than 15 countries of the continent and a crucial source of

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income for millions of rural people (Nnaemeka and Sun, 2021). Cotton as one of the most important economic crops provides more than 50% of the fiber source in the textile industry (Gao et al., 2019). Cotton stands as both a valuable fiber crop and an oil- producing crop on a global scale (Hongli et al., 2021) (Table 1).

In China and Africa, cotton farming has faced lot of setbacks, depriving them huge economy benefit from cotton and these has resulted to integration of genetic engineering approaches towards salvaging the challenges. Among other cotton species, the most abundant, largely cultivated and modified in China and Africa is *Gossypium hirsutum* Gh. Like other cotton producing continents, the majorly cultivated species of cotton is *Gossypium hirsutum* (Gh). Gh is also known as upland cotton or Mexican cotton considering its historical trace to Mexico. About 90% of all cotton production globally is of cultivars derived from this species. In tropical Africa, Gh is grown from sea-level up to 200 m altitude. Gh can also be grown on medium to deep, light to heavy well drained soils with a moderate fertility and a pH of 5-6 and 6-7.5 (-9.5) (Ikitoo, 2011). The lint of Gh is about 20 to 30 mm long in international market. Stem fiber cell of Gh investigated in Greece were about 0.8 mm long with a diameter of 18-20 um, a cell wall thickness of 3 to 4 um and a linen width of 12-13 um. The stems contained 40 to 44% a- cellulose and 13 to 18% lignin (Ikitoo, 2011). Also, the oil of Gh has shown antibacterial activity against Gram-positive and Gram-negative bacterial and the antibacterial activity was not affected by fermentation of the oil. Gh importance and demand contributed to it wide cultivation and interest.

COTTON FARMING IN CHINA AND AFRICA

In China and Africa, cotton is almost exclusively grown by smallholder farmers, and there are very few large plantations. The cotton plant loves warmth: It needs about 200 days of sunshine in the season to flourish and bear fruit. For that reason alone, it does well in the dry or humid Savannahs. China and Africa climate with its high average temperatures and alternation between dry and wet seasons favour the cultivation of this natural fiber crop. In many parts of the world, cotton is grown in large plantations, but in Africa the number of smallholder farmers cultivates cotton more than smallholder farmers in China. It is almost exclusively cultivated in Africa using crop rotation (Nkechi, 2020). That is, cotton is grown alternately with other crops such as basic food crops like maize, soybeans or groundnuts unlike China. The cultivation method imparted by cotton made in Africa also support smallholder farmers in growing food and this make an important contribution to food security. Artificial irrigation often used in large planting in China is practically unknown in Africa. Smallholder farmers in Africa practice rain fed cultivation, in other words they rely on natural rainfall being sufficient to water the crops. The wet and dry phases in agricultural part of China and Africa suit the cotton plant. In China, machine is use to harvest cotton but in Africa, harvesting is done mainly by hand picking. Hand pick cotton is also cleaner. However, in China and Africa cotton farming faced severe challenges due to pest attack. China and Africa have a climate favorable for pest growth and this result in sever attack of pests on cotton and subsequent yield losses.

Weather is one of the critical factors that affect insect breeding and movement. Positive physiological responses to increasing temperature allow for a faster insect population growth and facilitate movement. Most analysis by Weizhang et al. (2018) shows that in a warmer climate, pests may become more abundant and may expand their geographical range. Precipitation also affects crop-pest interactions. Both direct and indirect effects of moisture stress on crops make them more vulnerable to damage by pests especially in the early stages of growth. China and Africa climate also have warmed with stronger warming in the north and increased rainfall contrast between northeastern and Southern China. Weather effects on agricultural pests need to be assessed in the light of climate change which is projected to bring significant warming to large parts of China and Africa over changing decades.

INSECTICIDAL APPROACH TO COMBATING COTTON PEST ATTACK IN CHINA AND AFRICA

High population of insects attacking crops in China and Africa have been witnessed with insecticide application the only combating alternative as at that time. Despite growing evidence that *Bacillus thurigiensis* (Bt) cotton reduces use of insecticides, cuts farmers production costs and increase yields in the United States (Perlak et al., 2001), key countries that criticized biotechnology continue to doubt its usefulness, particularly for small farmers in developing countries. Examples of such countries as at that time include China (Pray et al., 2001) and South Africa (Ismael et al., 2001). This implies that China and Africa initially did not welcome Bt maybe for the argument that arose during that time that Bt cotton does not have any positive impact on yields and that bollworms are becoming a problem in China. Before the Bt invention, Chinese farmers have learned to combat this pest using pesticides. Initially, they used chlorinated hydrocarbons (DDT) until in the early 1980 (Stone, 1988).

In the mid - 1980s, farmers began to use organophosphates; however, in the case of cotton, pests developed resistance in the early 1990s, farmers began to use Pyrethroids, which were more effective and safer than organophosphates. Just like the case of other pesticides, China's bollworms began to rapidly develop

Table 1. Summary of production statistics for *Bt* cotton adopting country, 2009.

Country	Yield (M Kg)	Total ha (1000s)	% Bt	First Bt production
Argentina	181	430	70	1998
Australia	384	200	86	1996
Brazil	1252	836	14	2005
Burkina Faso	152	420	29	2008
China	7076	5300	68	1997
Colombia	30	38	64	2002
Costa Rica	0.2	1		2009
India	5117	10260	87	2002
Mexico	92	70	58	1996
South Africa	8	10	88	1997
United States	2654	3047	63	1996

Source: Adopted from Steven (2010).

resistance to Pyrethroids in the mid- 1990s. At this time farmers resorted to chemical cocktails or organophosphates, Pyrethroids and other chemicals (including DDT, although use of chlorinated hydrocarbons is illegal) with less and less impact on pests). With the rising pest population and increasing ineffective pesticides the volume of pesticides used by Chinese cotton farmers rose sharply. China became one of the largest pesticide consumers worldwide. An estimated 30 to 40% of all pesticides applied in China are used on cotton (Weizhang et al., 2018).

LIMITATIONS OF THE INSECTICIDAL APPROACH

Nearly 40% of the pesticides used by the Chinese cotton farmers contain active ingredients that are classified as extremely or highly hazardous by the World Health Organization contributing to around 400 to 500 cotton farmers death every year from pesticide poisoning. During the period of 1992-1996, the last five years for which aggregate data are available there was an average of 54,000 poisoning of farmers annually (Ferdaus et al., 2004). In addition, pesticides make their way to consumers as residues in fruits, vegetables and grains and through contaminated water supplies. It is clear in many countries that the use of pesticides which was the alternative carries many immediate as well as long term risks to human health. Farmers use more pesticides per hectare on cotton than on any other field crop in China (Huang Hu et al., 2002) and in the aggregate Chinese cotton farmers use more pesticides than farmers of any other crop with the exception of rice. Over all Chinese cotton production expands nearly US$500million on pesticides annually (Huang et al., 2002). Africa during this period was facing similar severe pests attack on cotton and was using only pesticides to combat this war with pests and most of the pesticides were imported from China. Majority of the imported goods in Africa are from China including pesticides. Thus, similar pesticide poisoning danger was posed to African cotton farmers. The use of pesticides against pest attack and its corresponding side effects in China and Africa have been in place for the past years. This adverse effect contributed to the choice made for alternative pest control approach which paved way to the integration of genetic engineering.

GENETIC ENGINEERING APPROACH

China pest problems led the nation scientists to pursue a variety of strategies including development of new pesticides, breeding of new pest-resistant cotton varieties and development of integrated pest management (IPM) programs for pest control with Africa sharply dependent on the best recommended approach after all. Consequently, when the possibility of incorporating genes for pest resistance came closer to reality, China's scientists became actively involved with funding primarily from the government research sources, a group of public research institutes led by the Chinese Academy of Agriculture Science (CAAS). China has devoted considerable resources into developing GM cotton expressing endotoxins from *Bacillus thurigiensis* (Bt) to control insect pests. The gene was transformed into major Chinese cotton varieties using China's own methods (Pollen tube pathways). The researchers tested the varieties for their impact on the environment and then released them for commercial use in 1997 (Pray et al., 2001). Monsanto in collaboration with the cotton seed company Delta and Pineland developed Bt cotton varieties that were approved for US commercial use in 1996. They began to collaborate with the Chinese National Cotton Research Institute of the CAAS at Anyang, Henan in the mid-1990s. In 1997, several

varieties were tested and approved by the Chinese Biosafety Committee for commercialization. Concurrently, scientists in the Cotton Research Institute were working on their own varieties. The research team began to release their varieties in the late 1990s (AgBioForum, 2002). As the adoption of Bt cotton spread, China's government research institutes at the province and prefecture levels produced new Bt varieties by backcrossing the Monsanto and CAAS varieties into their own local varieties. These varieties are now being adopted in Henan, Shandong and elsewhere. In the wake of commercialization of these approved and non-approved varieties, the spread of Bt cotton has been rapid. It has been estimated that farmers planted more than 2 million hectares of Bt cotton from 1996 to 2001 that is 45% of China's cotton growing area was planted with Bt cotton in 2001. In 1998 commercial production of Bt cotton by the Chinese farmers started in the Yellow River cotton producing region of Hebei, Shandong and Henan. Production rapidly expanded to 97% of the respective cotton growing areas in Hebei by 2000 and in Shandong by 2001. In Henan the adoption rate reached nearly 70% in 2001 (AgBioForum, 2002). The adoption rate was less in Jiangsu may be the cause of the observations during the field survey; the red spider mite problem was more serious than bollworm in their cotton production. In Hebei, Monsanto varieties were first approved. Genetically modified *Bacillus thurigiensis* Bt insect- resistant cotton and adoption progressed at different rates in different regions depending on the timing of Bt cotton varieties approved for commercialization and the availability of Bt cotton seed in local markets.

COMMERCIALIZATION OF BT COTTON

In China commercialization of genetically modified cotton (GMC) started in 1997; thereafter, a comparison of Bt cotton and non Bt was surveyed in terms of yield impacts, cost of production, farmer's income impacts, farmers health and environmental impacts. In all, Bt cotton has better positive result than the non-Bt. Although the spread of Bt cotton in China has relied on the varieties introduced by the public research system and seeds sold (at least initially) by the state-run seed network; the adoption of Bt varieties has been the result of decisions by millions of Chinese small farmers and is regulated by the government with less involvement of the private companies. The sustainability of GM crops has been subjected to heated debate in China but Bt cotton has proven less controversial as production, ecological and human health benefits have been realized and cotton is a non-food produce. The empirical evidence that GM technology offers long- term economic benefits than ecological benefits are realized, the economic benefits for Bt cotton exceeded US$5.3 billion over the years after it was commercialized (Yunhe et al., 2017). In China, Bt cotton had pervasive effects on the whole pest complex in cotton and its management adoption resulted in major reduction in insecticide use for bollworms control (Weizhang et al., 2018). Also, the 1999 and 2000 production survey (Pray et al., 2001) showed that Bt cotton continued to do well and increase yield in the northeast China (Yellow River) and central (Yantze River) cotton zone after the introduction and spread of Bt cotton using the nationally representative long panel data for 1997 to 2012. Fangbian Qiao showed that the economic benefit in China continues many years after the commercialization of Bt cotton. In West Africa precisely, approximately 25 to 35% of cotton yield is lost because of pest. The most important group of insects in terms of economic costs is the bollworm which causes discoloration of the cotton lint and automatically represents a serious decline in quality and substantial reduction in price. Aphids and bacteria blight (*Xanthomonas malvacearum*) are also examples of insects that affects cotton yield. These problems posed severe effect on the cotton industries in Africa and majority of them shutdown (Nnaemeka and Sun, 2021). Unfortunately, this has remained since that time and the total production remained far below the requirements of the textile and the oil mills. Although there are other challenges that contributed to the severe cotton production decline in Africa but pest infestation among others have greater significance damage. Restoration of the cotton glory in Africa has however become a subject of serious concern to cotton concerned Africans. There have been many improvements in the management of insect pests in cotton that have contributed to a reduction of insecticide use in this crop in the past two decades with perhaps the most notable being advances in biotechnology that have allowed engineering of plants to provide highly effective and selective control of caterpillar (order Lepidoptera) pests, the most significant pest group of cotton globally (Steven, 2010). Therefore, effort towards solving this problem was based on integration of transgenic cotton into cotton farming in Africa as was done in China etc. After the Bt discovery and application in China, China sister counterpart (Africa) and precisely South Africa as at that time followed through the processes and methodologies of the Chinese to develop and adopt the Bt cotton in same 1997. South Africa therefore became the first African country to adopt this technology after careful study of the Chinese success thus, bringing Africa to the map of Bt cotton farmers.

Therefore, *G. hirsutum* which is the most cultivated in Africa become genetically modified to resist the insects attack thus, the transgenic cotton currently in Africa consist mainly the Bt cotton. In Africa majority of the Bt cotton in use are produced by the Monsanto Company. The success of Bt cotton in Africa has been recorded for

instance Kenya use to produce 20,000 bales of cotton every year against a demand of 140,000 meaning they have to import the deficit. As the National Performance Trials for Bt cotton was completed with Bt they can produce up to “260,000 bales”. Like other countries, pests attack was the main reasons that caused cotton growing and textile industry shutdown, but Bt cotton which was adopted in 2012 after the constitution of the National Biosafety Council revitalized cotton farming in the country and only one variety named Seini (a Chinese variety) was released for commercialization (Nagala, 2013). In Sudan, Bt cotton out yielded the non Bt varieties more than 5 to 6 times evaluated in open field trials in six environments. Bt cotton contributed to a reduction in the damages caused by sucking insects and in the improvement of cotton quality by limiting stickiness. The cost of cotton insecticides and application for non Bt cotton cost $892 per hectare, reducing to $586 for Bt cotton, a saving of about 35%. The net profit for farmers for cultivating Bt cotton was estimated to reach $405 per hectare. South Africa began planting Bt cotton in 1998. The adoption rate continued to increase and the Bt cotton coverage reached 95% in 2007 (James, 2004). Bt cotton was adopted by large scale farmers and smallholders in South Africa. Besides the economic benefits, the number of insecticide sprayings related to the Bt cotton has decreased with a beneficial impact on the environment (Morse et al., 2006). In Burkina Faso, two regional BolgardII varieties were generated and commercialized in 2008, in collaboration with Monsanto (Vitale and Greenplate, 2014). In Nigeria, an economy rich country in Africa, her textile manufacturers association said in 2016 that “Genetically modified insect protected (Bt) cotton can play an immense role in restoring attraction to cotton farming as well as reviving and repositioning the textile sector in the country (Nnaemeka and Sun, 2021). The lack of confidence by participants across the cotton value chain over the years restricted the much-needed investment so it became the most important input industry “the cotton crop”, genetically modified insect protected (Bt) cotton” could improve cotton lint quality, farmers benefit and yields increase due to reduced insect pest damage; the release and commercialization of the Bt cotton could be related to successes recorded about the Bt cotton in other countries. Thus, in Nigeria the transgenic cotton was commercialized as the first genetically modified crop to boost the textile industry in 2018. Two home grown cotton varieties: MRC7377BGII and MRC7361BGII were developed by Mayhco Nigeria Private Ltd. in collaboration with the Nigeria Institute for Agricultural Research (IAR). Nigeria’s new Bt cotton was suitable for cultivation in all of Nigeria cotton growing zones and produced 4.1 to 4.4 tons per hectare compared to the local variety which yield just 600 to 900 kg ha^{-1}. In addition to the pest resistance traits, they offer early maturity, fiber length of 30.0 to 30.5 mm, fiber strength of 26.5 to 27. 0 g/tex (tenacity) and micronair (strength) of 3.9 to 4.1. The new varieties have saved farmers the trouble of contending with the local conventional variety, which is no longer accepted at the international market. Some other African countries have adopted Bt cotton as the transgenic cotton in use to combat pest infestation. Africa Bt cotton though developed in collaboration with foreign companies has proven safe, no negative impact on the environment and consumers. The Bollgard cotton reduced cotton production costs and insecticide use for the control of tobacco budworm (*H. virescens*) cotton bollworm (*H. zea*) and Pink bollworm (*P. gossypiella*) (Perlak et al., 1990, 2001). Agronomics trait, fiber quality and seed composition remain unchanged in the transgenic cotton. “Bt cotton after several food safety and risk assessment studies”, it is confirmable that Bt cotton hybrids pose no obvious toxic effects on non- target species. The analysis of Bt protein in the soil indicated that CryIAc protein is degradable without negative environmental impact. Further evaluation of the impact of GM protein leached by roots of GM cotton on the soil microflora showed that there was no significant difference in the population of microbes and soil invertebrates between both samples. Food safety assessment have shown that nutrient composition analysis of protein, carbohydrates, oil and calories also disclose no obvious difference in the Bt and non Bt cotton seed this was observed after comparison of animals fed with Bt and non Bt cotton seed thus, supporting the campaign that Bt cotton is as safe as the conventional cotton in almost every ramification (Nnaemeka and Sun, 2021). Nigeria Bt cotton success and Nigeria decisions to commercialize Bt cotton has revived hopes for the novel variety in Ghana (Gakpo, 2018) as well as other African countries. Nigeria’s green lightening of Bt insect resistant cotton spurred Africa’s increase interest acceptance of GMO’s (Steven, 2010).

The reduction in pesticides use due to the adoption of Bt cotton in China and Africa has been substantial. However, Africa cannot currently be equally compared to China as Africa depends on China for so many things including Agriculture. It cannot be stated however that Africa has learned useful lessons from China which they are integrating in their economy and agricultural policy. Advances in the Bt cotton commercialization was made and currently it is estimated that Bt cotton is covering about 60% of the total Chinese cotton area but one can consider that the coverage is close to 100% wherever the target pests of the Bt cotton are a real threat (Michael and Naiyin, 2007).

LIMITATIONS OF THE GENETIC ENGINEERING APPROACH

Despite tremendous improvement in breeding and other

technologies for robust yield, increase crop pests remain an important cause of considerable yield loss triggering the use of insecticides that once affected farm profit and the health of human and their environment (Weizhang et al., 2018). Cornell University researchers at the American Agricultural Economics Association (AAEA) annual meeting in Long Beach, Calif, July 25 reported that other pests are now attacking the GM cotton in China. Although Chinese cotton growers were among the first farmers worldwide to plant genetically modified (GM) cotton to resist bollworms, the substantial profits they have reaped for several years by saving on pesticides is now been eroded because population of other insects such as Mirids have increased so much that farmers are now having to spray their crops up to 20 times a growing season to control them according to the study of 481 Chinese farmers in five major cotton producing provinces. The problem in China therefore is not due to the bollworm developing resistance to the Bt cotton as some researchers feared but is due to secondary pests that are not targeted by the Bt cotton which previously have been controlled by the broad -spectrum pesticides used to control bollworms. Furthermore, the practice of applying excessive amount of highly toxic pesticides has continued even after the adoption of Bt cotton in China and Africa, perhaps as a result of lack of knowledge and some behavioral factors by the farmers following reoccurrence of secondary pest in China and pest development of resistance to Bt cotton in Africa as seen in Burkina Faso. As late comers in the Bt technology, this reoccurrence may also be viewed as lack of clear understanding of the pests by the farmers because when US farmers plant Bt crops they unlike farmers in Africa required by contract with seed producers to plant a refuge, a field of non- Bt crops to maintain a bollworm population nearby to help prevent the pest from developing resistance to the Bt cotton. The pesticides used in these refuge field help control secondary pest populations on the nearby Bt cotton field (Pinstrup-Anderson, AAEA, 2007). This observation from US practices has inspired further efforts in China and Africa. Wang et al. (2016) said that one of the solutions to eradicating secondary pests attack is also by introducing natural predators to kill the secondary pests or enforcing the planting of refuge areas where broad- spectrum pesticides are used.

THE *BACILLUS THURINGIENSIS* CRYSTAL STRUCTURE AND BIOCHEMISTRY

Bt stands for the naturally occurring bacterium (*Bacillus thuringiensis*). Bt is a ubiquitous Gram- positive rod – shaped and sporulating bacterium that has been isolated worldwide from a great diversity of ecosystems including soil, water, dead insects, dust from silos, leaves from deciduous trees, diverse Conifers and insectivorous mammals as well as from human tissues with severe necrosis (Koller et al., 1992,). Bt strain synthesizes crystal (Cry) and cytolytic (Cyt) toxins at the onset of sporulation and during the stationary growth phase as parasporal crystalline inclusions (Du et al., 1994). In the past decades, more than 700 cry genes sequence that codes for crystal (Cry) proteins have been identified and the large plasmids appear to be the usual location of these genes. The crystal proteins are aggregates of proteins that builds up to form crystal. Cry protein are encoded by Cry genes. Apart from the crystal, other Bt isolates have various functions such as attack on human cancer cells apart from the known insecticidal properties (Ekino et al., 2014). Since the identification and cloning of the first Bt insecticidal crystal protein gene in 1981, the number of genes coding for novel insecticidal proteins has continuously increase namely: CryI for proteins toxic for Lepidopterans, CryII for proteins with toxins against Lepidoptera and Dipterans, CryIII for proteins toxic for Coleopterans, CryIV for proteins exclusively toxic for dipterans. About 73 different types of Cry are known (Cry1-Cry73) (Ekino et al., 2014). In addition, other criteria can be used to identify Cry apart from base on the target insect. Currently the Cry proteins constitute the largest group of insecticidal proteins produced by species of *Bacillus.* Within the decade, Cry genes with specificity for different groups of insects have been cloned and sequenced. Regarding the Cry toxins, at least 5 different groups not related in their sequence has been characterized (Soberon et al., 2018). In addition to the Cry toxins, Bt contains transposomes (transposable genetic element that flanked genres and that can be excised from one part of the genome and inserted elsewhere). All these properties increase the variety of toxins produced naturally by Bt strains and provide the basis for commercial companies to create genetically engineered strains with novel toxin combination (Jim Deacon, 2001). The different strains available is because there can be up to 5 to 6 different plasmids in single Bt strain and these plasmids can encode different toxin genes and the plasmids can be exchanged between Bt strains by a conjugation-like process, thus, paving way for potential wide variety of strains. By the mid-1970s, about 13 Bt strains effective against Lepidopterans had been identified and classified according to their Cry genes. With different combinations of Cry toxins (Jim Deacon, 2001) the crystalline parasporal inclusions (CPI) produced by the Bt is usually composed of one or several polypeptide subunits which are toxic when ingested by susceptible insects. These CPI contain proteins that exhibit a variety of biological actions including cytolytic, hemolytic and entomocidal activities (Aronson et al., 1986, Hofte and Whiteley, 1989). Numerous natural variations in the primary structure of the crystal proteins exist and are responsible for differences in susceptible

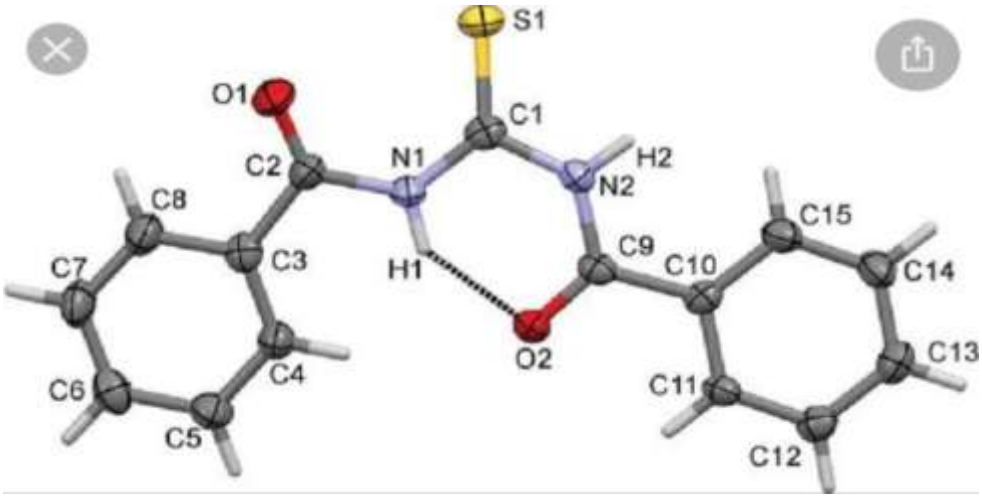

Figure 1. Crystal structure of the Bt compound with selected atoms labeled (Ekino et al., 2014).

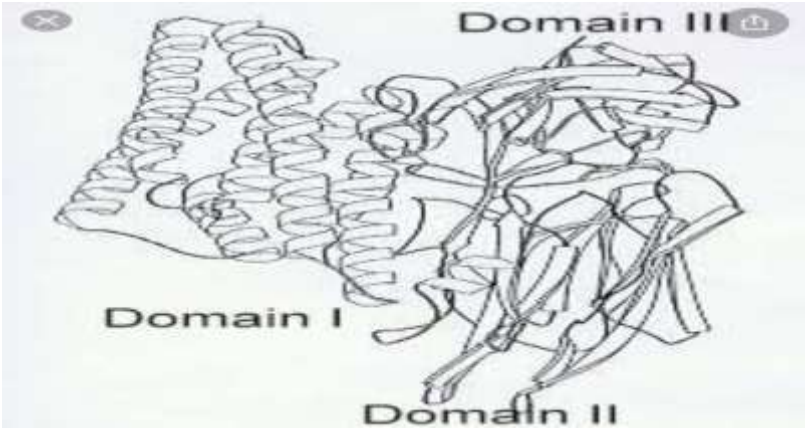

Figure 2. *Bacillus thuringiensis* toxin (Schnepf et al., 1998).

host range of each toxin (Hofte and Whiteley, 1989). Many physiochemical properties of the crystal inclusions have been reviewed (Huber and Luthy, 1981, Koller et al., 1992, Du et al., 1994) including the pH required for solubilization of the crystal an important parameter, since it is an essential step for toxicity in susceptible insects. The Bt Cry proteins comprise at least 50 subgroups with more than 200 members (Figure 1). The members belong to a three-domain family and the larger group of Cry protein is globular molecules with three structural domains connected by single linkers (Ekino et al., 2014).

A common characteristic of Cry genes is that they are expressed during the stationary phase growth. Cry proteins, the end-products of Cry gene expression constitute 20 to 30% of the cell dry weight and generally accumulate in the mother cell (Bravo, 2007). The highest level of Cry gene synthesis appears to be coordinately controlled by a variety of mechanisms occurring at the transcriptional, posttranscriptional and post translational levels. Several Cry gene promoters have been identified and their sequence determined (Figure 2).

BT GENE MODIFICATION HISTORY AND METHODS

This new technology for managing insect pests in China and Africa was approved for commercialization in the United States by the US Environmental Protection Agency in 1995 and is currently available in many other countries. The Bt cotton variety presently used against tobacco budworms, bollworms and certain other caterpillars attacking cotton produce the CryIAc protein. Therefore, a cotton plant is modified to produce Cry

protein within the plant tissues from which the insects can eat it that is the Cry protein traits is carried in plants genes as is traditional plant resistance to insects. During the development process, biotechnology created Bt cotton by inserting selected exotic DNA from a Bt bacterium into the cotton plants own DNA. Following the insertion of modified Bt DNA into the cotton plant DNA, seed companies moved the Cry proteins trait into high performance cotton varieties by traditional plant breeding methods meanwhile agronomics qualities for yield, harvestability, fiber quality and other important characteristics were preserved at the same time the Cry proteins gene was added to commercial varieties. The three primary components of the genetic package inserted into cotton DNA include protein gene, promoter, and genetic marker.

This genetic package can be inserted into cotton plant DNA through a variety of plant transformation techniques. Transformed plants may be affected by the genetic package as well as the location of the new genes in the plant DNA. The insertion site may affect the Bt protein production and other plant functions as well. Therefore, biotechnology companies carefully scrutinized each transformation to ensure adequate production of Bt protein and to limit possible negative effects on agronomic traits. Following a successful transformation, plants are entered into traditional backcross breeding program with the variety chosen to receive the foreign Bt gene package. The final product of a Bt cotton variety is developed after four or five backcross generations. Although the new transgenic Bt cotton variety, agronomic qualities can be considerably different. This process of making Bt cotton was adopted from the United States Department of Agriculture, Agricultural Research Service ARS-154, January 2001 (Hardee et al., 2001). Several companies can now make Bt cotton.

However, out of a number of strategies for insect resistance management, three are key: (i) Achieving high toxin dosage either by the use of strong promoters or by targeting the protein to organelles or by tissue specific expression of the protein (ii) use of multiple genes preferably, those that work through different mechanisms and (iii) use of a refuge along with (i) and (ii) (Singh et al., 2016).

BT GENE EXPRESSION LEVELS

In Singh et al. (2016) experiment, the Cry protein expression resulting from this experiment was observed only in the green plant parts. No transgenic protein expression was observed in the non-green parts including roots, seeds and non-green floral tissues. The result of this experiment also showed that a transgenic protein having the transit peptide of a protein that accumulated in green plastids does not get targeted to the leucoplasts. Thus, applying such a transit peptide could be an effective method of expressing a transgene- encoded protein only in green aerial tissues. These features also allay public concern about the safety of Bt cotton since it expression level in the seed is low.

Also, Allah et al. (2012) stated that the production of transgenic plants with stable high- level transgenic expression is important for the success of crop improvement programs based on genetic engineering. In their study they evaluated genomic integration and spatio temporal expression of an insecticidal gene (Cry2A) in pre-existing transgenic lines of cotton. Genomic integration of Cry2A was evaluated using various molecular approaches. The expression levels of Cry2A were determined at vegetative and reproductive stages of cotton at regular intervals. Gene expression was found variable at various growth stages as well as in different plant parts throughout the season: The leaves of transgenic were found to have maximum expression of Cry2A gene followed by squares, bolls, anthers and petals. The protein level in fruiting part was less as compared to other parts showing inconsistency in gene expression. Expression level also varies with age of the plant. Spatio temporal study by Greenplate et al. (1998) reveals that expression level of Cry2A declined during the crop growth with toxin level falling to 15 to 20 nanogram per gram of fresh tissue weight. That is young plants tend to show higher expression than the older plants. The reduction in Bt protein contents in late-season cotton tissues could be attributed to the over expression of the Bt gene at earlier stages which leads to gene regulation at post-transcription levels and consequently results in gene silencing at a later stage (Allah et al., 2012). Although, the mechanisms of variation in endotoxin protein content in plant tissues are rather complicated, the level and efficiency to which genes are expressed are mainly regulated by their cis-regulatory elements such as the promoters.

BT GENE EXPRESSION RESISTANCE MAINTAIN AND LOSSES

A major weakness of the products (Bollgard I and II) currently used in the field is a drop in the Cry1Ac protein's expression level as the plant matures and sets bolls. Furthermore, there is a high expression level in the roots that provides no resistance against *H. armigera* and other lepidopteran pests, as they do not feed on roots. Another weakness is that a secondary lepidopteran pest on cotton can survive the low Cry1Ac protein dose present in the developing bolls and leave progeny (Singh et al., 2016). As is already known, Tobacco budworms and bollworms are not the only insect pests that attack cotton and unfortunately, the CryIAc protein has essentially no effect on many of the secondary pest such

as *Pectinophora gossypiella*, boll weevils, cotton aphids, cotton fleahoppers, cutworms, spider mites, Stink bugs, tarnished plant bugs, and white flies. In some caterpillar species Bt may provide only 10 to 50% control. Research has suggested that Bt cotton insecticidal protein is not expressed steadily (Knox et al., 2006) due to a number of factors: Soil water deficit significantly affects insecticidal protein expression in the leaves of Bt cotton (Rochester, 2006; Parimala and Muthuchelian, 2010). Increased damage to Bt cotton by cotton worm in Shandong and Hebei provinces of China in 2005 and 2006 may have been due to lack of rain and a resulting soil water deficit from June to July (Liu et al., 2008). Likewise, Carter et al. (1997) and Benedict et al. 1996) found that lack of rain resulted in soil water deficit and associated water stress reduced the content of total soluble protein and insecticidal protein in June and July. Drought stress could lead to DNA degradation in cotton seedling tissues, producing many residual DNA fragments that could inhibit the synthesis of functional proteins and structural proteins (Yang et al., 2016). Thus, several lines of independent evidence implicate drought stress in the failure of insect resistance of Bt cotton. In most of the world, drought is an important problem during the cotton whole growing period (Li et al., 2010). Environment is also an important factor that contributes to influence insect resistance of Bt cotton in a number of ways. One hypothesis suggested that under an adverse environment, DNA methylation of the promoter regions of the Bt gene switches off gene expression (Stam et al., 1997). Another hypothesis suggested that tannin, generated by cotton plants exposed to adverse environments, was binding to Bt insecticidal protein and inactivating it (Holt, 1998). A third hypothesis suggested that the protein synthesis decreased, resulted in decreased Bt insecticidal protein content (Chen et al., 2005). However, how soil water deficit affects the expression of Bt insecticidal protein in bolls, and what mechanism is responsible for these effects has not been efficiently reported. Methylation of the promoters may also play a role in the declined expression of endotoxin proteins (Singh et al., 2016).

Fitness is also one of the key parameters to evaluate the effects of transformed plants on the ecological environment (Liu et al., 2020). Bt cotton growing in different habitats (farmland, grassland and shrubs) were assessed for fitness level. It was found that the expression of Bt protein in the farmland was significantly higher than that in the other habitats (Liu et al., 2020).

Theoretically, Bt cotton may also indirectly lower the general abundance of some beneficial insects. It is commonly known that more than 500 species of insects and mites have developed at least some degree of resistance to insecticides (Georghion and Wirth, 1997). Most scientists therefore, agree that the tobacco budworm and the bollworm will eventually become resistant to the CryIAc protein used in current Bt cotton varieties someday due to a number of predicted factors. Hardee et al. (2001) also stated that before exposure to Cry toxins by planting Bt cotton the very few tobacco budworms and bollworms (Perhaps 1 in 100,000 or 1 in 1 million) carry two copies of a resistance allele (RR) meaning they are fully resistant to Bt cotton while some have a single copy of a resistant allele and a susceptible allele (RS), meanwhile the overwhelming majority have two copies of a susceptible allele (SS). Most of the (SS) are killed after feeding on Bt cotton depending on the dose of Cry toxin in the plant. The (RS) usually are more difficult to kill than the (SS) still the (RS) are not considered Bt resistant in most cases but (RR) are not killed. This perhaps is why Bt cotton cannot achieve 100% eradication of the insect pest. A clear evidence of pests developing resistance to Bt cotton have been seen in China and Africa (Burkina Faso precisely) where there was reoccurrence of the insect pest in the cotton farm after a period of time and this nearly demoralized the use of Bt transgenic cotton in China and in some part of Africa if not for the precaution measures taken by Nigerians during their Bt cotton development, the future of Bt cotton in Africa may have been impossible to predict as a result of the pest resistance observed in Burkina Faso. The resistance has been traced to the (RR) species because after the introduction of Bt cotton in China and Burkina Faso; non-target pests became more abundant due to less pesticide that were sprayed followed by the gradual pests resistant to the toxin. Regardless, improvements in the Bt cotton technology continue towards completely eradicating any insect attacking cotton (Figures 3 and 4).

CONCLUSION

Cotton farming in China and Africa has shown fluctuation in quantity over the years: Less quantity before Bt technology, greater quantity with Bt technology and lesser quantity again after secondary pest and target pests developed resistance. The Cry gene use in making Bt cotton in China and Africa have shown variable expression with the nucleotide sequence of the gene, promoters and the insertion point of the gene in the DNA of the transgenic variety, transgene copy number, the internal cell environment as well as several external factors in the environment (Guo et al., 2001) being responsible. Prompting investigation at molecular, genetic as well as physiological levels with the aim of understanding the differential expression of transgenes and the quantitative changes in insecticidal proteins in insect resistant cotton plant which has been known to have beneficial impact on global cotton farming due to the reduction in the number of pests and hence the total application of chemical insecticides used for it control as well as the final production especially in China and Africa

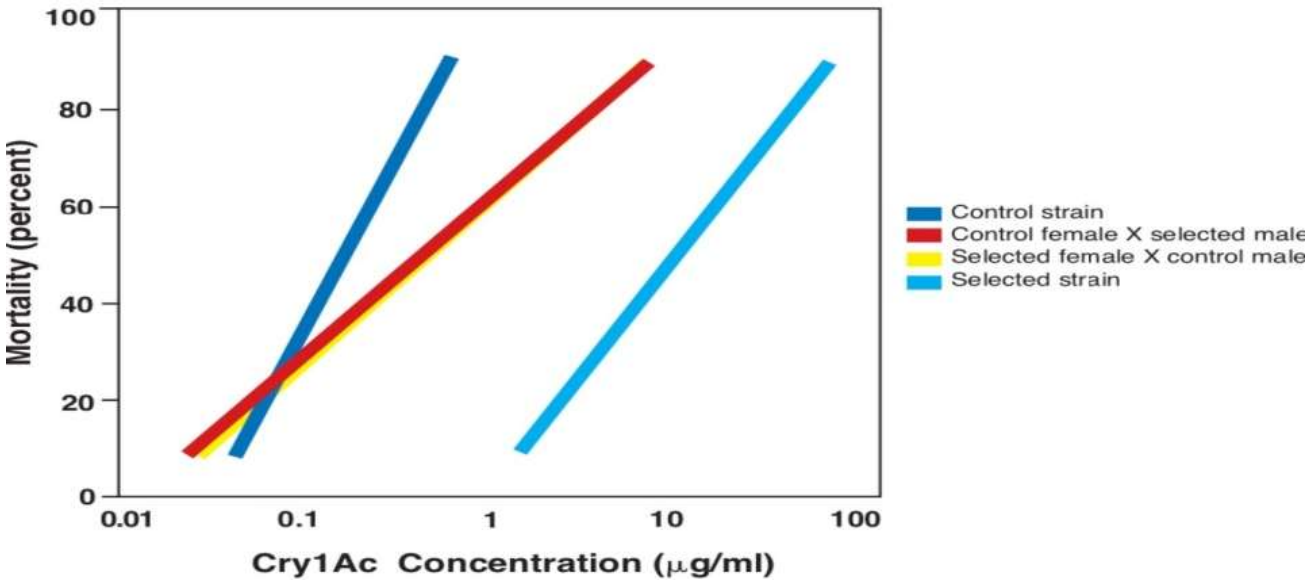

Figure 3. Development of resistance to Bt cotton in tobacco budworm in laboratory experiment has been found (Gould et al., 1992).

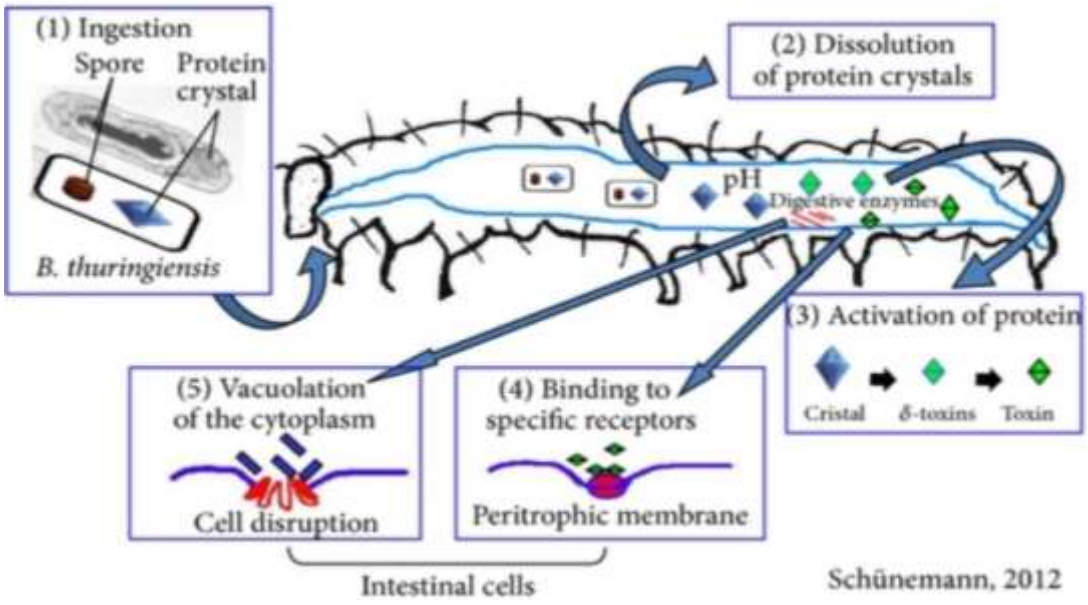

Figure 4. Mode of action of *Bacillus thuringiensis* in Lepidoptera (Rogerio et al., 2014).

where insects infestation is high but low pest management practices mainly in Africa. Following this investigation, it is vital to ensure efficient expression of the insecticidal protein in Bt cotton through genetic engineering and the utilized mechanisms should be well understood in other to plan rational resistance management strategies to slow the rate at which insects develop resistance and to control target pests effectively by enhancing endotoxin expression through genetic or agronomic management. Because this technology has faced serious challenges especially in the quality of the cotton fibers, abundant growth of non-target pests and the insects developing resistance to it, China and Africa growers should therefore know that production costs can

increase as insects develop resistance to the Bt toxin and the Cry toxins in Bt plants are not easily replaced when insect develop resistance, developing new transgenic insecticidal crop is also difficult, time consuming and cost intensive. Thus, preserving the effectiveness of Bt cotton is one way to keep pest management costs at the lowest level and this can be further achieved by producing a high dose of Bt plant Cry toxins throughout the season; effective IRM refuges must be maintained, using vegetable insecticidal proteins (VIPs) and using genes from plants or animals which encode immunosuppressive proteins are recommended to enable continuation of transgenic plant and precisely transgenic cotton farming in China and Africa without future setbacks. Finally, developing new cotton varieties with more powerful resistance, applying certain plant growth regulators and maintaining general health of the transgenic crop are substantial in realizing the full transgenic potential in transgenic Bt cotton in China and Africa. Development of new promoter that will induce more consistent production of insecticidal genes throughout the life of the cotton plant and also throughout the plant part especially the fruiting parts that are also susceptible to attack should not also be excluded in the advancement for effective Bt cotton. Integration of these recommendations can go a long way in sustaining Bt technology in China and Africa

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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