African Journal of Agricultural Research

Academic Journals

ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, post harvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer-reviewed.

Contact Us

African Journal of Agricultural Research

ARTICLES

Full Length Research Paper

Effect of a dietary supplementation combining a probiotic and a natural anticoccidial in broiler chickens

Redha DJEZZAR[1], Karima BENAMIROUCHE[2], Djamila BAAZIZE-AMMI[3], Ramdane MOHAMED-SAID[2] and Djamel GUETARNI[2]*

[1]Higher National Veterinary School of Algiers, Algeria.
[2]Department of Biology, Faculty of Nature and Life Science, University of Blida 1, Algeria.
[3]Institute of Veterinary Science, University of Blida 1, Algeria.

In order to improve the growth performance of broilers and prevent coccidiosis in our farms, two groups of broiler chicks (Hubbard F15) were bred under the same conditions for a period of 52 days. "Experimental group" received an aliment added with a natural anticoccidial based on herbal extracts and a probiotic but water free of antibiotics while "Control group" received the same food without probiotic and natural anticoccidial, but added with a chemical anti-coccidial and a water containing antibiotics. The obtained results showed, in support of the subjects belonging to the "experimental group", a difference in weight without bearing statistically significant difference, higher consumption rates accompanied by a low mortality rate and a length of upper intestines. The enumeration of oocyst excretion showed a marked increase, characterized by three peaks corresponding to three episodes of coccidiosis in the "control group" and a much smaller increase without clinical expression in the "experimental group". The autopsy of the animals sacrificed in the "experimental group" showed the total absence of clinical coccidiosis lesions unlike those performed to the subjects in the "control group" who presented a final average lesion score of 3.5 in D_{22}, 3.8 and 3.2 respectively, on D_{30} and D_{45}, confirming the recurrence of coccidiosis. The weight of the plucked and eviscerated carcasses and of the edible offal of the chickens who consumed food supplemented with probiotics and natural anticoccidial based on herbal extracts are superior to the weight of the subjects belonging to the "control group".

Key words: *Pediococcus acidilactici*, *Yucca schidigera*, *Trigonella foenum-graecum*, supplementation, broiler, feeding, zootechnical performance.

INTRODUCTION

The antibiotics stand among the most common additives used to improve feed efficiency, growth rate and consequently increase the productivity and profitability of poultry farms. However, they favored the emergence of antibiotic residues in the food chain, a large number of resistant animal bacterial strains (Ungemach et al., 2006) and allergic reactions of the consumers, as well as failures of the antibiotic treatment on humans (Corpet,

1996; Mathlouthi et al., 2009). The desire to maintain a satisfactory level of production requires the research of non-therapeutic alternatives to substitute antibiotics as growth factors that should be equally effective from the zootechnical, health and economical point of view. Among the proposed additives we include the enzymes, the organic acids, the natural plant extracts, the probiotics and prebiotics (Dorman and Deans, 2000).

Probiotics are able to control the carrying and dissemination of pathogens and zoonotic agents and can also contribute to potentiate the food and consequently the profitability of farming (Trufanov et al., 2008; Niderkorn et al., 2009). Several studies have shown, in addition to the zootechnical efficiency (Simon et al., 2001; Vittorio et al., 2005), their beneficial effects on the health of poultry (Awaad et al., 2005; Vandeplas et al., 2009; Higgins et al., 2010). The improvement of the zootechnical performance by orientation of the flora has been reported by many authors (Cabuk et al., 2004; Alfaro et al., 2007); nevertheless, the results regarding their efficacy are not consistent (Zhang et al., 2005). Our recent works (Djezzar et al., 2012) have shown that the use of *Pediococcus acidilactici* (Bactocell MA 18/5M strain) certainly allowed some improvement of the zootechnical performance, but its effectiveness remains limited due to the problem of coccidiosis, a major and recurrent pathology in broilers breeding.

Coccidiosis, the most common parasitic disease to be found in poultry farming, causes considerable economic losses due, in part, to the massive mortality rate in their acute and very dangerous form, as hidden in their chronic or subclinical and weaken the health status of animals, predisposing them to other bacterial and viral diseases (Naciri et al., 2005). The endogenous development of parasites may be limited by the use of food additives or by stimulating immune defenses by making use of anticoccidial vaccines. The coccidiostats (synthetic products or ionophores) are facing increasing resistance of coccidia (Reperant et al., 2012). The herbal extracts rich in steroidal sapogenins, especially *Yucca schidigera*, have various biological activities acting preemptively against the coccidial risk. In Algeria, the means to fight against coccidiosis confine to the use of chemical anticoccidials in food and drinking water. Vaccination remains absent due to its high cost (Alloui and Barberis, 2012).

The purpose of this study is to test the effectiveness of a dietary supplementation combining a probiotic (*P. acidilactici*) and an anticoccidial based on herbal extracts (*Y. schidigera and Trigonella foenum graecum*) in our local breeding conditions

MATERIALS AND METHODS

Animals, feed and treatments

Five hundred and twenty, 1 day-old chicks of *Gallus gallus domesticus* belonging to the Hubbard F15 strain, mixed sexes,

homogeneous weight, coming from the same hatchery, were divided into two groups (n=260) with five repetitions of 52 subjects each. They were put in place in January 2012 to be raised under the same breeding conditions for a period of 52 days, in a traditional building, partitioned as to provide ten areas of life, 6 m^2 each, undergoing the same environmental conditions.

The floury-type food used, based on sweet corn, soybean cake, wheat bran, di-calcium phosphate, calcium and vitamin-mineral concentrates was produced especially for our experiment, based on a formula taking into account the three breeding phases [starting (D_1-D_{28}), growing (D_{29}-D_{42}) and finish (D_{43}-D_{52})].

The animals in the "experimental group" received a drinking water free of additives and an aliment supplemented with the anticoccidial "Yuquina XO$^®$" (NOR-FEED, South France) based on the herbal extracts of "*Y. schidigera and T. foenum graecum* "at a rate of 0.5 kg/t and lyophilized *P. acidilactici* CNCM MA18/5M (Bactocell ®, France) at a rate of 100 ppm (concentration of 10^9 CFU/g), while those subjects belonging to the "control group", were administered the same dietary aliment, free of probiotic and herbal extracts, but supplemented with a chemical anticoccidial (Robenidine, Cycostat) and water rich in antibiotics, the treatments most frequently administered on the Algerian field throughout the breeding period.

The subjects of the two groups were vaccinated on D_6 against Newcastle disease (UNI L CEVA ®), the vaccination being repeated on D_{15} and D_{19} against Gumboro disease (IBD L CEVA ®).

Zootechnical parameters and intestinal morphometry

The average live weight was calculated by weighing 200 subjects (starting phase) and 100 subjects (growing and finish phases). Feeds distributed for control and experimental groups were weighted at the end of each breeding phase (D_{28}, D_{42} and D_{52} of age) to calculate feed conversion ratio.

Death cases were recorded daily and mortality rates were determined at the end of each breeding phase (D_{28}, D_{42} and D_{52}). We have not recorded death cases suffered during the first three days due to transport stress.

The intestinal morphometry was performed on 10 subjects from each group, on D_{28}, D_{42} and D_{52}. After anesthetic treatment, the animals were sacrificed by bleeding and the length of the intestine as a whole (from the duodenum gizzard-junction up to the distal end of the colon) was measured.

Oocysts excretion and intestinal lesion scores

A 20 g sample of fresh droppings, issued on litter, is collected daily from the five areas from each batch during the period (D_{13} to D_{48}). The oocysts, contained in 5 g of droppings of each sample were analyzed by the method of Mc Master, according to concentration, by flotation in a dense saturated solution of magnesium sulfate (density: 1.3) (EUZEBY 1981, 1987). The average number of oocysts is expressed per gram of droppings (o.p.g).

The lesion scores were determined based on the autopsy of five subjects taken from different locations in the building and sacrificed, at the first suspicious signs of coccidiosis (diarrhea and death), according to the method of Johnson and Reid (1970) as amended by Bouhelier (2005).

The research of the lesions was carried out systematically at the autopsy of all fresh mortalities.

Carcass yield

To evaluate carcass yield and perform the intestinal morphometry(*) at the end of the breeding cycle (D_{52}), 10 subjects were

randomly selected from each group, previously fasted (for 12 h), were individually weighed, sacrificed by bleeding and plucked. After cold storage (+8°C) for a period of 12 h, the carcasses - head and legs were first cleared and then reweighed. After evisceration, the edible organs (gizzard, heart and liver), the abdominal fat and carcass were systematically collected and weighed separately.

Statistical analysis

The statistical analysis was performed based on the test of homogeneity applied on two means of two populations ("experimental" and "control" groups). We used the hypotheses test (H_0 and H_1) based on the calculation of the critical ratio (CR) on the sample database which is compared to the value of the table of the normal distribution with threshold value $\alpha = 5\%$.

Formulation of hypothesis: H_0: $\mu_1 = \mu_2$ and H_1: $\mu_1 \neq \mu_2$

Sampling distribution is a Student distribution because standards deviations are unknown. There are estimated from the samples data. The Student's distribution is approximated by the Gauss distribution because size of sampling is greater than 30; then $t(\alpha/2, n_{1-1} + n_{2-1}) \approx z\alpha/2$.

By default, the signification's level $\alpha = 5\%$ brings us to compare the statistically calculated (Zcal) to the tabulate value $z\alpha/2 = 1.96$.

$$Z_{cal} = \frac{|\bar{X}_1 - \bar{X}_2|}{\sigma_{x_1 + x_2}} \text{ with } \sigma_{x_1 + x_2} = \sqrt{\frac{S_1^2}{n_1} + \frac{S_1^2}{n_2}}$$

If Zcal < $z_{\alpha/2}$; then hypothesis H_0 is accepted: the two population means are homogeneous.

RESULTS AND DISCUSSION

Zootechnical parameters

The results of the zootechnical and morphometric parameters obtained at the end of each breeding phase are reported in Table 1. The results showed a difference in weight between the subjects of the control and experimental groups (CG and EG) at the end of breeding (2678 g *vs* 2791 g, respectively), but no statistically significant difference ($\alpha=5\%$). The positive effect of this probiotic on growth has been demonstrated in fattening pigs, broilers and laying hens by Awaad et al. (2003), Vittorio et al. (2005), Chevaux et al. (2006), Di Giancamillo et al. (2008), Alkhalf et al. (2010) and Abd-El-Rahman et al. (2012).

The best feed conversion ratio, achieved by the subjects of the "experimental group" versus the ones achieved by the subjects in the "control group", counting for the three phases of breeding (1.32 *vs*. 1.48: starting, 1.57 *vs*. 1.73: growing and 2.39 *vs* 2.83: finish) could find an explanation based on the positive effect of lactic acid bacteria on feed efficiency, which was reported by Jin et al. (1998) and Simon et al. (2001).

We recorded a high rate of mortality in the "control group" as compared to the "experimental group" (14.7% *vs*. 6.5%). The situation on the high mortality observed in the "control group" appears to be consistent with the development of coccidiosis on D_{18} (10.1%: starting). It could be a result of the low level of the anticoccidial in the food. As for the low mortality recorded by the "experimental group", it appears to be the result of anticoccidial based on herbal extracts, introduced preventively.

The average length of the intestines of chickens supplemented with probiotics is significantly higher than those having received the food without additives at the end of the three phases of breeding. This increase reaches 10% (p<0.001) on D_{28}, approximately 7% (p<0.001) on D_{42} and 15% (p<0.05) on D_{52}. According to Samli et al. (2007), *Enterococcus faecium* NCIMB 10415 increases the weight gain, the conversion rate and the size of the villi in the ileum.

Oocyst excretion and lesion scores of the coccidiosis

Enumeration of oocysts

Average value of o.p.g. for each day and group during $D_{13} - D_{52}$ period is graphically represented in Figure 1. The obtained results show a pronounced increase and statistically significant oocyst excretion in the "control group" characterized by three peaks on D_{19-24}, D_{30} and D_{45} corresponding to three episodes of coccidiosis. This ascertainment is confirmed by the appearance of blood in faeces (Photo 1) strengthening our hypothesis regarding the anticoccidial under-dosage used, or the potential resistance of coccidia. In the experimental group, the excretion is much smaller and appears with a slight delay (D_{25} and D_{37}).

However, it should be noted that the sharp decreases in oocyst excretion observed in the control group are consistent with the administration, on D_{22} and D_{30} of sulfonamides (Coccidiopan®) and on D_{45}, of a chemical anticoccidial (Toltrazuril, Baycox®). These treatments may have reduced the gap between the zootechnical performance in experimental group and control group.

Lesion scores of coccidiosis

Upon observation of mortality cases (D_{22}, D_{30} and D_{45}) with onset of diarrhea (Photo 1) on the litter of the control group, the autopsy of the animals sacrificed from the two groups revealed the average final lesion indexes reported in Table 2.

The autopsy of the animals sacrificed in the experimental group showed no clinical coccidiosis lesion (Photo 2a) during the entire breeding period (scores below 2). On the contrary, the subjects in the control group showed pathognomonic signs of coccidiosis (Photo 2b). The mean lesion score of 3.5 obtained on

Table 1. Zootechnical and morphometric parameters.

Groups	Parameters	End of the three phases of breeding		
		D_{28}	D_{42}	D_{52}
"Control"	Average live weight by subject (g)	996 ± 23	1802 ± 31	2678 ± 29
	Feed ratio	1.48	1.73	2.83
	Mortality rate (%)	10.1	13.4	14.7
	Average length of the intestines (cm)	191 ± 13	245 ± 15	295 ± 14
"Experimental"	Average live weight by subject (g)	1011 ± 27	1778 ± 25	2791 ± 27
	Feed ratio	1.32	1.57	2.39
	Mortality rate (%)	2.5	3.7	6.5
	Average length of the intestines (cm)	212 ± 17	262 ± 15	345 ± 14
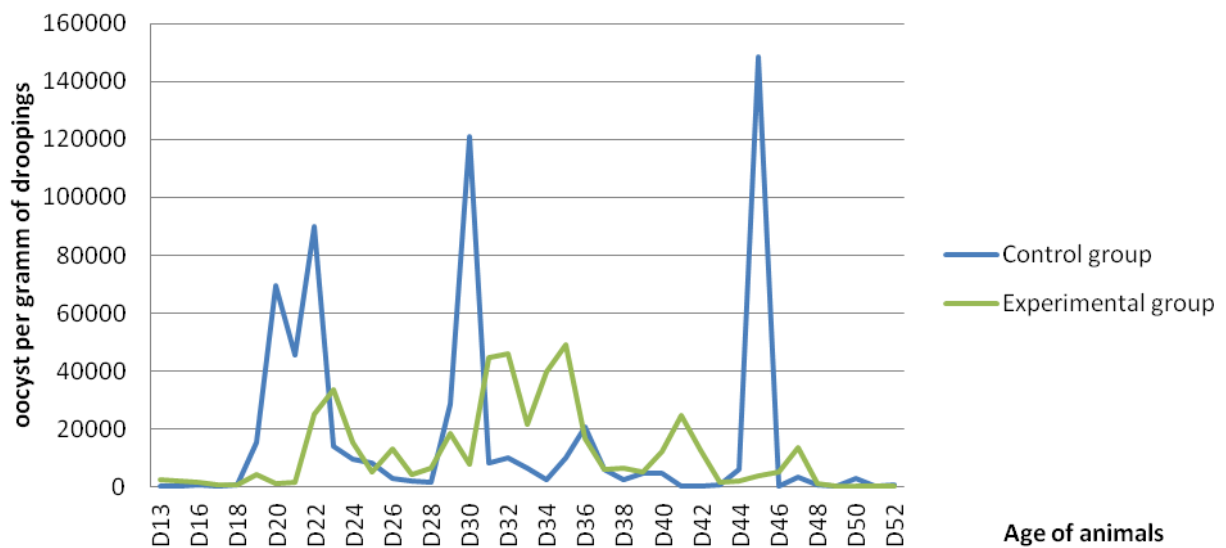
Figure 1. Kinetics of oocyst excretion in Control and Experimental Groups.
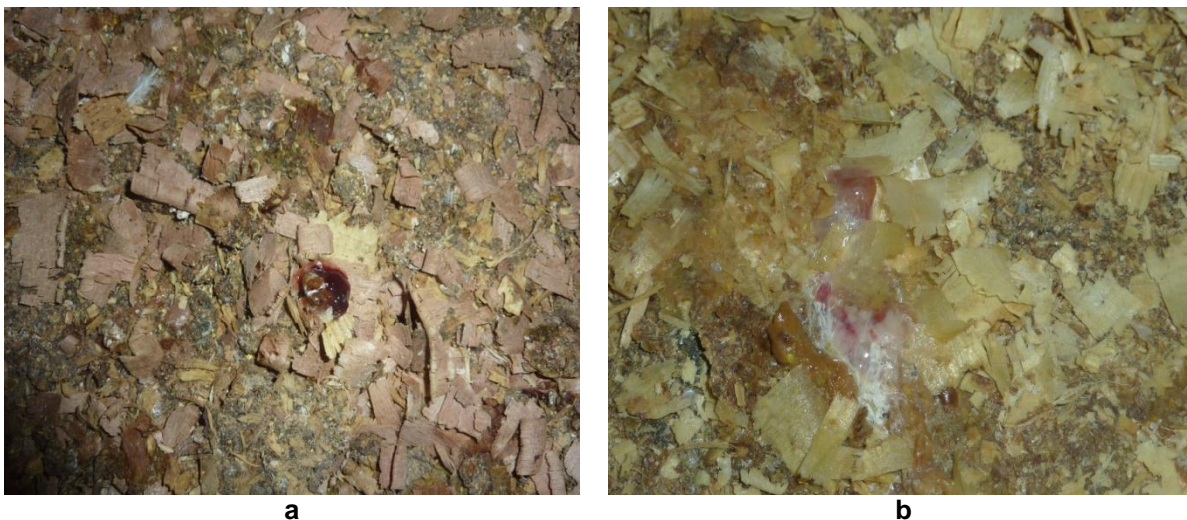
Photo 1. Presence of bloody droppings on litter of "control group" at (a) D_{22} and (b) D_{30}.

Table 2. Average final lesion indexes obtained for the two groups at the end of each breeding phases.

Groups	End of each breeding phases		
	D$_{22}$	D$_{30}$	D$_{45}$
Control	3.5	3.8	3.2
Experimental	1.5	1.7	1.1

(a)   (b)

Photo 2. Lesions observed at the autopsy of sacrificed subjects, for Experimental group (a) Punctate congestion duodenum (D$_{24}$) and Control group (b) Caeca hemorrhagic (D$_{30}$).

D$_{22}$ revealed the first episode of clinical coccidiosis, those of 3.8 and 3.2 obtained on D$_{30}$ and D$_{45}$ respectively and confirmed the recurrence of coccidiosis.

Autopsy of fresh cadavers

The autopsy of the fresh cadavers from the "experimental group" revealed the presence of punctate congestions scarcely scattered in the duodenum in two sporadic cases on D$_{24}$ and tracheitis in a sporadic case on D$_{35}$ (Photo 3a and b). However, the autopsy of the subjects of the Control group revealed the presence of pericarditis associated with a perihepatitis translating into a colibacillosis complication in two sporadic cases on D$_{22}$ and the presence of macerated blood at the level of the intestines and caeca marking the clinical episodes of coccidiosis on D$_{22}$, D$_{30}$ and D$_{45}$ (Photo 3c and d).

Carcass yield

The average weights and yields of carcasses obtained at the end of breeding (D$_{52}$) are reported in Table 3. We clearly see that the weight of the plucked and eviscerated carcasses is higher in the chickens that have been administered a diet supplemented with probiotics and herbal extracts. The weight of the edible offal (gizzard, heart and liver) of the experimental group is higher than those of the control group; as to the abdominal fat, the weight difference between the two groups was not significant.

It could be mentioned that the association "probiotics and herbal extracts" does not induce excess abdominal fat as compared to the Control group which is likely to endorse the weight of the eviscerated carcass (the abdominal fat being removed at the slaughterhouse). Indeed, anatomical differences were highlighted between the animals (broilers) fed either with an aliment made of wheat (D+) or with food made from corn-soybean (D-). The pro-ventricle and gizzard are more developed in D+, by way of contrast, the small intestine is more developed in D- (Peron et al., 2006; Garcia et al., 2007; Rougiere et al., 2009; Rougiere and Carré, 2010). Rougiere and Carré (2010) were also able to highlight the food retention time in the gizzard and significantly longer pro-ventricles in D+ relative to D-, whereas at intestinal level, no difference was visible.
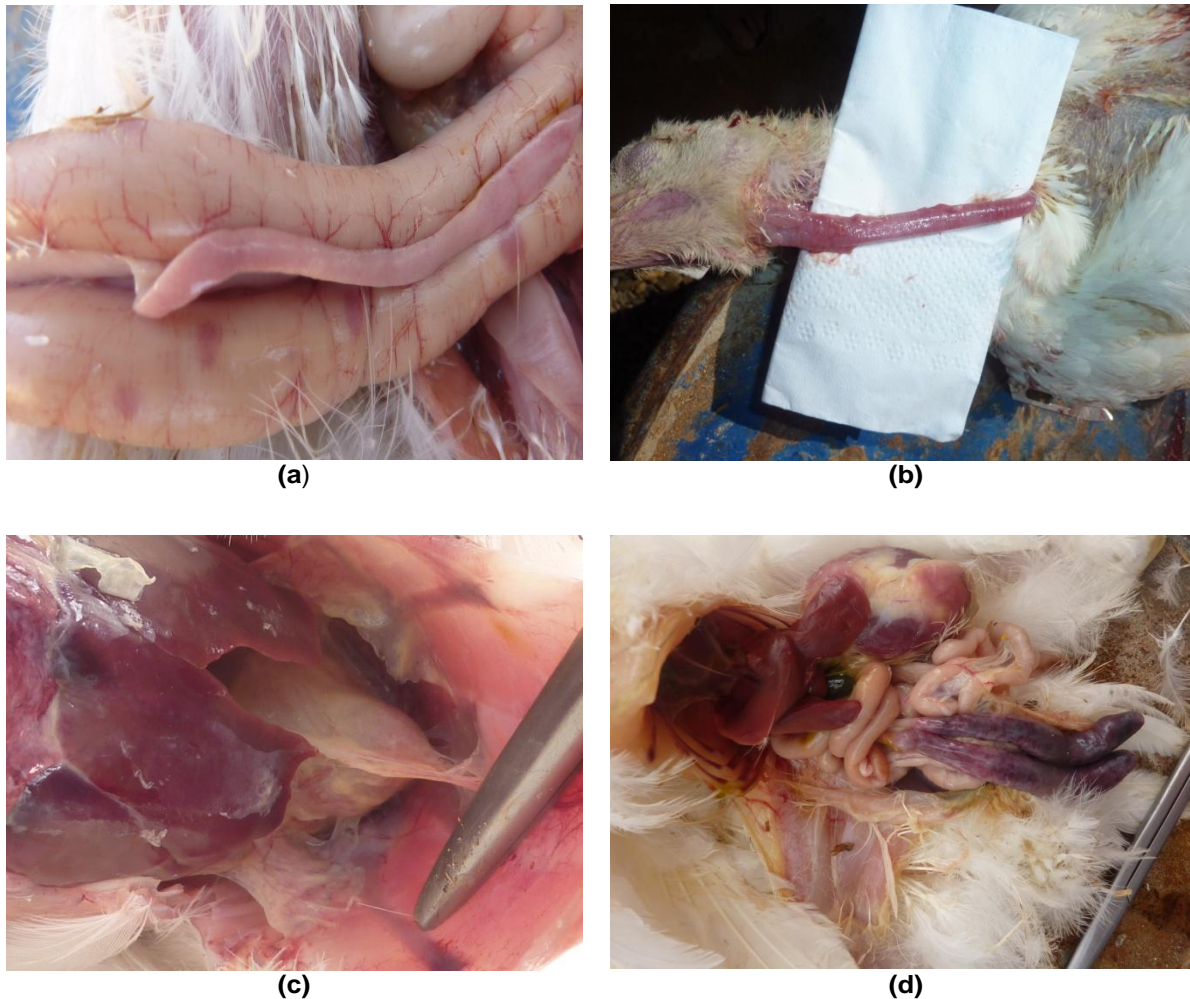
Photo 3. Lesions at autopsy of fresh cadavers from the two groups, viz: Experimental group (a) Punctate congestions of the serous (D_{24}) (b) Tracheitis (D_{35}); Control group (c) Pericarditis and perihepatitis (D_{27}) and (d) Presence of blood in the ceaca (D_{45}).

Table 3. Average weights and carcass yield.

Groups	Weight (g) of ($\bar{x} \pm SEM$)						Carcass yield (%)
	Liver	Plucked carcasses (*)	Eviscerated carcasses	Grease	Edible offal	Intestines	
Control	2678 ± 29	2182 ± 153	1784 ± 131	42 ± 11	77 ± 20	279 ± 27	66.6
Experimental	2791 ± 27	2423 ± 137	1930 ± 127	37 ± 9	105 ± 11	351 ± 36	69.2

(*): carcasses to which have been removed the head and legs.

Conclusion

The probiotic *P. acidilactici*, used alone or in combination in poultry feed, increases the gain of live weight, meat, edible offal and size of the intestines without causing an excess of abdominal fat. It improves the dietary efficiency by acting favorably upon the balance of the intestinal flora of chickens.

The low oocyst excretion and the absence of clinical signs of coccidiosis observed in the subjects belonging to the experimental group could be the result of the effectiveness of anticoccidial based on herbal extracts *(Y. schidigera* and *T. foenum graecum*).

Faced with the alarming situation of excessive use of

anticoccidial (antibiotics and other chemicals) in poultry breeding, this biological product based on herbal extracts, requiring no waiting time, could stand as a real alternative product.

The combination of these biological products would, in addition, allow us maintain a satisfactory level of production, address the issues related to other antibiotics resistance and anticoccidials, preserve the quality of chicken meat (drug residues) and consequently, consumers' health.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

Abd-El-Rahman AH, Kamel HH, Ahmed WM, Mogoda OSH, Mohamed AH (2012). Effect of Bactocell® and Revitilyte-Plus as Probiotic Food Supplements on Tm the Growth Performance, Hematological, Biochemical Parameters and Humoral Immune Response of Broiler Chickens. World Appl. Sci. J. 18(3):305-316.

Alfaro DM, Silva AVF, Borges SA, Maiorka A, Vargas S, Santi E (2007). Use of *Yucca schidigera* extract in broiler diets and its effects on performance results obtained with different coccidiosis control methods. J. Appl. Poult. Res 16:248–254.

Alkhalf A, Alhaj M, Al-homidan I (2010). Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi J. Biol. Sci. 17(3):219-225.

Alloui N, Barberis (2012). Effet de l'association d'un prébiotique et d'un anticoccidien sur la santé du poulet. 10èmes Journées des Sciences Vétérinaires, 27 & 28 mai, Ecole Nationale Supérieure Vétérinaire, Alger.

Awaad MHH, Afify MA, Zouel-Fakar SA, Shalaby B, Chevaux E, Delforge J, Dussert L, Khettou M (2005). Effets de l'addition de *Pediococcus acidilactici* sur l'infection à *Escherichia coli* et sur la colonisation par *Clostridium perfringens* et *Salmonella typhimurium* chez le poulet de chair. Sixièmes Journées de la Recherche Avicole, St Malo pp. 502-505.

Bouhelier B (2005). Prévalence des coccidies en élevages de poulet sous label rouge du Gers. Etude expérimentale. Thèse pour l'obtention du grade de vétérinaire. Université Paul Sabatier de Toulouse (France).

Cabuk M, Alcicek A, Bozkurt M, Akkan S (2004). Effect of *Yucca schidigera* and natural zeolite on broiler performance. Int. J. Poult. Sci. 3:651-654.

Chevaux E, Skiba F, Granier C, Moreau R, Le Treut Y (2006). Effect of *Pediococcus acidilactici* (MA/18/5M) supplementation on piglets digestibility and growth performance. Abstract in Proceedings of the 19th IPVS Congress, Copenhagen, Denmark pp. 42-43.

Corpet DE (1996). Microbiological hazards for humans of antimicrobial growth promoter use in animal production. Rev. Med. Vet. 147:851–862.

Djezzar R, Benamirouche K, Baazize-Ammi D, Khoubei A, Merrouki A, Maghni E, Guetarni D (2012). Impact of dietary supplementation with *Pediococcus acidilactici* on zootechnical and sanitary performances of broilers in Algeria. Res. J. Poult. Sci. 5(4-6):54-59.

Dorman HJD, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88:308–316.

Euzeby J (1981). Experimental diagnosis of animal helminths: pets, animals, primates; helminthology of veterinary labs: Book 1 general, Ante-mortem diagnosis P. 347.

Euzeby J (1987). Protozoologie médicale comparée. Fondation Mérieux édition pp. 262-257.

Garcia V, Gomez J, Mignon-Grasteau S, Sellier N, Carre B (2007).

Effects of xylanase and antibiotic supplementations on the nutritional utilisation of a wheat diet in growing chiks from genetic D+ and D-lines selected for divergent digestion efficiency. Animal 1:1435-1442.

Di Giancamillo, Vitari F, Savoini G, Bontempo G, Bersani V, Dell'Orto C, Domeneghini VC (2008). Effects of orally administered probiotic *Pediococcus acidilactici* on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. Histol. Histopathol. 23:651-654.

Higgins SJP, Higgins E, Wolfenden AD, Henderson SN, Torres-Rodriguez A, Vicente JL, Hargis BM, Tellez G (2010). Effect of lactic acid bacteria probiotic culture treatment timing on *Salmonella Enteritidis* in neonatal broilers. Poult. Sci. 89:243-247.

Jin LZ, Ho YW, Abdullah N, Jalaludin S (1998). Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poult. Sci. 77:1259-1265.

Johnson JJ, Reid WM (1970). Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30-36.

Mathlouthi N, Kechiche MS, Elleuche W, Larbier M (2009). Les parois de levures : facteurs de croissance et alternatives aux antibiotiques chez le poulet de chair. Huitièmes Journ. Rech. Avicole St Malo, 25 et 26 mars. 2009:40.

Naciri M, Fort G, Picaud T, Recoquillay F (2005). Etude de l'efficacité de deux formules d'extraits végétaux EMX1 et EMX2 dans la prévention des coccidioses à *E. Acervulina* et *E Tenella* du poulet label. Proceedings des 6èmes Journ. Rech. Avicole pp. 384-388.

Niderkorn V, Morgavi DP, Aboab B, Lemaire M, Boudra H (2009). Cell wall component and mycotoxin moieties involved in the binding of fumonisin B1 and B2 by lactic acid bacteria. J. Appl. Microbiol. 106(3):977-985.

Peron A, Gomez J, Mignon-Grasteau S, Sellier N, Besnard J, Derouet M, Juin H, Carré B (2006). Effects of wheat quality on digestion differ between the D+ and D- chicken lines selected for divergent digestion capacity. Poult. Sci. 85:462-469.

Reperant JM, Dardi M, Pagès M, Thomas-Hénaff M (2012). Pathogenicity of *Eimeria praecox* alone or associated with *Eimeria acervulina* in experimentally infected broiler chickens. Vet. Parasitol. 187(8):333-336.

Rougiere N, Carre B (2010). Gastric retention is a key determinant for differences between chickens from D+ and D− genetic lines selected for divergent digestion efficiency. Animal 4:1227–1239.

Rougiere N, Gomez J, Mignon-Grasteau S, Carre B (2009). Effects of diet particle size on digestive parameters in D+ and D− genetic chicken lines selected for divergent digestion efficiency. Poult. Sci. 88:1206–1215.

Samli HE, Senkoylu N, Koc F, Kanter M, Agma A (2007). Effects of *Enterococcus faecium* and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Arch. Anim. Nutr. 61(1):42-49.

Simon O, Jadamus A, Vahjen W (2001). Probiotic feed additives – effectiveness and expected modes of action. J. Anim. Feed Sci. 10:51-67.

Trufanov OV, Kotyk AM, Bozhok LV (2008). Effect of probiotic preparation based on *Bacillus subtilis* (BPS-44) in experimental mycotoxicoses of chickens. Mikrobiol Z. 70(1):52-58.

Ungemach FR, Müller-Bahrdt D, Abraham G (2006). Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. Int. J. Med. Microbiol. 296(2):33-38.

Vandeplas S, Dubois Dauphin R, Thiry C, Beckers Y, Welling GW, Thonart P, Théwis A (2009). Efficiency of a *Lactobacillus plantarum*-xylanase combination on growth performances, microflora populations, and nutrient digestibilities of broilers infected with *Salmonella Typhimurium*. Poult. Sci. 88:643-1654.

Vittorio SA, Mauro F, Carla B, Giovanna DD, Giovanni S, Chevaux E (2005). Effets de l'addition de *Pediococcus acidilactici* dans la ration de poulets de chair sur les performances zootechniques et la microflore intestinale. St Malo (France) 6èmes Journ. Rech. Avicole pp. 208-211.

Zhang AW, Lee BD, Lee SK, Lee KW, An GH, Song KB, Lee CH (2005). Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult. Sci. 84:1015-1021.

Full Length Research Paper

Effect of organic fertilizer (cocoa pod husk) on okra and maize production under okra/maize intercrop in Uhonmora, Edo State, Nigeria

Gerald O. Iremiren[1] and Rotimi R. Ipinmoroti[2]*

[1]Department of Crop Science, University of Benin, P. M. B. 1154, Benin-City, Edo State, Nigeria.
[2]Soils and Plant Nutrition Section, Cocoa Research Institute of Nigeria, P. M. B. 5244, Ibadan, Oyo State, Nigeria.

The study determined effects of cocoa pod husk (CPH) fertilizer rates on okra and maize performance in okra/maize intercrop at Uhonmora, Edo State, Nigeria. Four levels of fertilizer: 0, 1.0, 2.0 and 4.0 t ha^{-1} and seven cropping patterns: okra sole at 0.60×0.75 m, 0.90×0.75 m, 1.20×0.75 m, sole maize at 0.3×0.75 m, okra intercropped with maize at 0.60×0.75 m, 0.90×0.75 m, 1.20×0.75 m with 1, 2 and 3 maize stands respectively between 2 okra stands for 28 treatments in a split plot design in three replicates. Fertilizer resulted to significant ($p \leq 0.05$) increased okra and maize yields that were optimum at 0.90×0.75 m with CPH applied at 1.0 t ha^{-1}. The land equivalent ratio (LER) values were higher than unity in okra/maize intercrop but the aggressivity values were negative when okra was planted at 1.20×0.75 m, which indicates unhealthy competitions between the component crops. The monetary values (MV) were optimum when okra was planted at 0.90×0.75 m and was recommended.

Key words: Cropping patterns, economic value, growth factors, optimum revenue, soil fertility maintenance.

INTRODUCTION

Maize (*Zea mays*) ranks third after sorghum and millet in importance in human food and livestock feed supply among the cereal crops in Nigeria (Uzozie, 2001) while okra is among the foremost fruit vegetable crops in terms of production and consumption level (Iremiren and Okiy, 1999). Okra fruits are popular health food materials with high fibre, Ca, K, vitamin C, foliate and anti-oxidant contents. The seed contains about 15% oil which is high in unsaturated fats such as oleic and linoleic acids (Yadev and Dhanker, 2002). However, the vegetative and reproductive growths of the crops are affected by spacing, soil fertility, species of weeds and climatic factors (Iyagba et al., 2012).

Late flowering and late maturing genotypes of West Africa okra with long growth cycle are common in Nigeria farming practices (Adeniji, 2007). One of the late maturing West African okra accessions is *Abelmoschus caillei* [A. Chev] Stevols which is commonly called *Ila Iroko* in southwestern Nigeria. It has well-developed root system, good drought and heat tolerance as well as tolerance to root-knot nematodes. Though late maturing, it has sweet and spineless light-green pods. The plants

Table 1. Okra/maize plant population ha^{-1} in okra/maize cropping systems.

Cropping pattern	Okra stands ha^{-1}	Maize stands ha^{-1}
Sole okra at 0.60 m x 0.75 m	22,222	-
Sole okra at 0.90 m x 0.75 m	14,814	-
Sole okra at 1.20 m x 0.75 m	11,111	-
Sole maize at 0.30 m x 0.75 m	-	44,444
Okra/maize at 0.60 m x 0.75 m	22,222	22,222
Okra/maize at 0.60 m x 0.75 m	14,814	29,628
Okra/maize at 0.60 m x 0.75 m	11,111	33,333

are 5 ft high, well-branched with an open growth habit (Bamire and Oke, 2003).

Okra production in Nigeria is of two distinct seasons namely - the thick and lean seasons. The lean season falls toward the dry season when late maturing okra varieties are common, hence okra fruits are produced in low quantities, scarce and expensive (Bamire and Oke, 2003). Majority of okra producers/growers in Nigeria plants okra on a small-scale probably due to the problem of small land holdings, unavailable of storage, processing and preservation facilities (Agbede and Kalu, 1995). Processing and preservation are carried out using traditional techniques of slicing, sun-drying and grounding using mortar and pestle (Farinde et al., 2007). Hence, okra production is common on less than 0.4 to 1.6 ha and grown in intercrops with maize, yam, cassava, pepper, pineapple, maize and yam, yam and pepper, cassava and pepper, cassava and beans, beans and maize amongst other combinations depending on locality (Bamire and Oke, 2003; Iken and Amusa, 2004; Raji, 2007).

Studies have been carried out on sole okra and maize as well as their intercrops as influenced by plant density for early maturing cultivars of okra (Iremiren et al., 2007, 2013; Iyagba et al., 2012) but not yet on the late maturing cultivars either as sole or as in okra-maize intercropping system. The experiment was conducted to investigate the influence of cocoa pod husk fertilizer on late maturing okra and maize production in okra-maize intercropping system under different maize densities and okra plant spacing on the field at Uhonmora, Edo State, Nigeria.

MATERIALS AND METHODS

A field plot of 60.2 x 18.5 m was cleared and divided into 3 blocks each of 17.4 x 14.5 m with 2.0 m distance between blocks. The blocks were each made into 28 flat beds of 7 rows with 4 beds row^{-1}. Each flat bed was 3.6 x 1.5 m size with 1.0 m space between the beds within the rows. There was 1.0 m space between rows of beds and 2.0 m edge row space round the entire plot.

Late maturing okra variety (*Abelmoschus caillei* [A. Chev] Stevols) seeds were planted in 3 rows bed^{-1} as sole at (1) 0.60×0.75 m, (2) 0.90×0.75 m, (3) 1.20×0.75 m and as intercrop with maize at (4) 0.60×0.75 m with 1 maize stand between 2 okra stands, (5) 0.90×0.75 m with 2 maize stands between 2 okra

stands, (6) 1.20×0.75 m with 3 maize stands between 2 okra stands and (7) sole maize at 0.3×0.75 m for a total of 7 cropping patterns. The 7 cropping patterns were each randomly allocated to the 7 rows of 4 beds each. Where okra or maize was planted, 4 seeds of the crop were sown and thinned to one plant stand^{-1} 2 weeks after sowing. Cocoa pod husk (CPH) was collected, dried, pulverized and composed for 21 days (Iremiren et al., 2007) as organic fertilizer and applied at 0, 1.0, 2.0 and 4.0 t ha^{-1} across the 7 rows of 4 beds each, with each bed having a rate of CPH application to give a 7×4×3 factorial in split plot design, with cropping patterns as main plot and KPH rates as sub-plot. The plant population in each cropping pattern is given in Table 1. The first cropping trial commenced on 30th April, 2010, while the second cropping trial commenced on 5th May, 2011. The two cropping trials were under rain fed condition.

Hoe weeding was carried out bi-monthly and harvesting of maize was carried out after 120 days of planting. Harvested maize cobs were sun dried, shelled and the grains dried to 10% moisture content and weight (g plant^{-1}) taken. Okra fresh fruit harvest (g plant^{-1}) commenced 27 weeks after sowing and continued every 5 days for 15 cumulative harvests, when harvests became negligible. The data for both maize and okra were averaged for the mid row plants and extrapolated to yield ha^{-1}, which excluded the border plants of each bed per treatment. Data were statistically analyzed by ANOVA and significant mean differences separated by LSD at 5% probability. Productivity evaluation of okra/maize intercrop compared to their sole cropping was assessed using the land equivalent ratio (LER) (Mead and Wiley, 1980), aggressivity (McGilchrist, 1965) and the monetary values (MV) (Gomez and Gomez, 1983) was calculated using the current farm gate price of ₦300 kg^{-1} for maize and ₦150 kg^{-1} for okra.

Pre-planting soil sample was analyzed for pH using glass electrode pH meter at soil/water 1:2.5 ratio; particle size distribution was by hydrometer method, available P was by Bray P-1 method (Bray and Kurtz, 1945); N by micro Khjeldal method (Bremmer, 1996); organic C by Walkey-Black wet oxidation method (Nelson and Sommers, 1982). The exchangeable cations were determined by extracting the soil with 1.0N NH$_4$AOC at pH 7.0 (Tel and Rao, 1982). The K was measured using flame photometer while Ca and Mg were by atomic absorption spectrophotometer (AAS). The CPH was analyzed for N and organic C as described for the soil. The K, Ca and Mg content was by AAS after ashing the CPH sample in Murphy furnace at 500°C and the ash dissolved with 4% HCl and made to volume with distil water. The rainfall data of the study location in 2010 and 2011 were taken.

RESULTS

Soils and fertilizer material

The study site soil was sandy clay loam in texture and
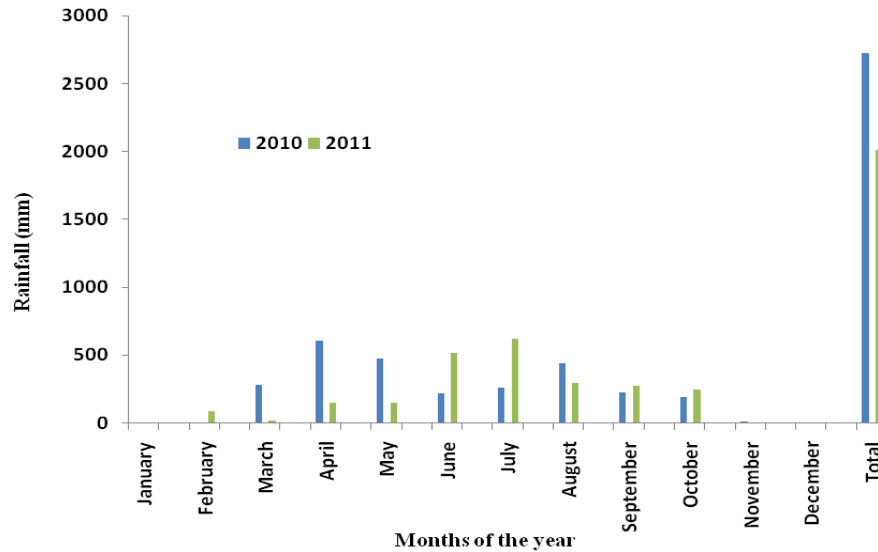
Figure 1. Rainfall distribution for 2010 and 2011 at Uhonmora, Edo State, Nigeria.

contained moderate amount of organic C, total N, available P and Ca with very low K and Mg contents (Table 2). The soil was slightly acidic with pH value of 5.4. The cocoa pod husk contains considerable amount of N, P, K, Ca and Mg with C:N ratio of 16.6.

Rainfall data

The rainfall data showed that rainfall commenced late in 2010 but stopped late in the month of November, while it started earlier in 2011 and stopped in October. However, there was more rain in 2010 than recorded in 2011 (Figure 1). The amount and distribution of the rainfall during the cropping periods were more even in 2010 than in 2011.

Okra fruit yield

Okra fruit yield was 867.8 to 4622.2 kg ha^{-1} in 2010 cropping season, while it was 845.9 to 4216.4 kg ha^{-1} in 2011 cropping season (Table 4). The okra fruit yield increased with increasing okra plant stands ha^{-1} in both sole and intercropping systems. The okra fruit yield was higher under okra/maize intercropping system compared to values obtained under sole okra cropping (Table 3). The fruit yield values were higher with the application of CPH than for the control for both years of cropping. The values were 867.8 to 2217.8 kg ha^{-1} for control and 2105.5 to 4622.2 kg ha^{-1} for CPH applied okra in 2010, while it was 845.9 to 1850.2 kg ha^{-1} for the control and 2017.5 to 4216.4 kg ha^{-1} for CPH applied okra in 2011. Thus, weight of okra fruits obtained increased with CPH application rate and the values were more for 2010

compared to 2011.

Maize grain yield

Maize grain yield was 3.40 to 5.67 t ha^{-1} for 2010, while it was 3.71 to 5.96 t ha^{-1} for 2011. The maize grain yield increased with maize population ha^{-1} at both cropping years (Table 4). On cocoa pod husk fertilizer application, maize yield was 3.40 to 4.63 t ha^{-1} for control and 3.56 to 5.67 t ha^{-1} with CPH application in 2010, while it was 3.71 to 4.60 t ha^{-1} for control and 3.87 to 5.96 t ha^{-1} with CPH application in 2011. The grain yield generally increased with rate of CPH application. Under okra/maize intercropping pattern, maize grain yield increased with rate of CPH application up to 2 Mt ha^{-1} application rate and then decreased, while under sole maize, the maize grain yield increased from 4.63 to 5.67 t ha^{-1} at 1.0 t ha^{-1} of CPH application and thereafter decreased with increased CPH application (Table 4).

Maize grain yields were generally higher in 2011 cropping season than for 2010. Cocoa pod husk application resulted to 4.13 to 27.96% increase in maize grain yield over the control. The maize grain yield under sole maize cropping was 0.87 to 31.71% higher than those under okra/maize intercrop. The mean differences between the cropping patterns and CPH application rates for both 2010 and 2011 cropping seasons were significant at p≤0.05.

Productivity evaluation of okra/maize intercrop

The yields of okra and maize in the okra/maize intercrop compared to their sole cropping resulted to land

Table 2. Some properties of site soil and cocoa pod husk (CPH) organic fertilizer used.

Material s	pH	Organic C	N	P	K	Ca	Mg	Sand	Silt	Clay
Soil		← g/kg →		mg/kg	← cmol/kg →			← g/kg soil →		
	5.4	32	1.0	7.2	0.3	2.56	0.05	67.6	14.2	18.2
Cocoa pod husk		← %				→				
	-	24.3	1.46	0.15	3.96	0.8	0.3	-	-	-

Table 3. Fresh okra fruit yield (kg ha^{-1}) under different cropping pattern and cocoa pod husk fertilizer.

Cropping pattern	Cocoa pod husk application rate (t ha^{-1})			
	0.0	1.0	2.0	4.0
	← Fresh okra fruit yield (kg ha^{-1}) →			
2010 cropping				
Okra/Maize at 60 cm	2217.8	3173.3	3377.8	3515.5
Okra/Maize at 90 cm	1760.5	2394.8	2105.5	2470.1
Okra/Maize at 120 cm	867.8	2884.4	2664.4	2532.2
Sole okra at 60 cm	1532.2	2902.2	3835.5	4622.2
Sole okra at 90 cm	1887.9	3254.3	2978.3	3017.2
Sole okra at 120 cm	896.8	2340.0	2508.9	2784.4
LSD = 0.05	100.2	131.6	109.1	264.7
2011 cropping				
Okra/Maize at 60 cm	1850.2	2975.8	2895.5	3281.4
Okra/Maize at 90 cm	1711.8	2402.1	2217.2	2403.8
Okra/Maize at 120 cm	871.3	2732.6	2583.6	2433.5
Sole okra at 60 cm	1346.1	2900.4	3183.6	4216.4
Sole okra at 90 cm	1698.4	2984.1	2597.1	2889.7
Sole okra at 120 cm	845.9	2092.7	2017.5	2142.5
LSD = 0.05	152.5	125.2	211.6	207.8

LSD = Least significant difference.

equivalent ratio (LER) values that were greater than unity with value range of 1.39 to 2.18 in 2010 and 1.68 to 2.39 in 2011 cropping seasons respectively (Table 5). This culminated to land area of about 28.1 to 54.1% in 2010 and 40.5 to 58.2% in 2011 being saved. The aggressivity values calculated due to intercropping of the late maturing okra variety with maize were positive at okra spacing at 60×75 cm and 90×75 cm, while it was negative for okra spaced at 120×75 cm.

The monetary value (MV) was higher under okra/maize intercrop than either of okra or maize cultivated separately (Table 6). The maize component of the okra/maize intercrop contributed more than okra to the calculated monetary values. On the overall, the monetary values were highest under okra spacing at 120×75 cm with 3 maize stands between two okra stands. However, the aggressivity values calculated for okra/maize at 120 cm x 75 cm spacing were all negative (Table 6), while it

was positive for okra/maize intercrop at 90×75 cm and 60×75 cm okra spacing.

The combined monetary values for 2010 and 2011 cropping seasons showed the superiority of okra + maize in okra/maize intercrop over sole cropping of okra and maize. The okra/maize intercrop was optimal at the 90×75 cm okra spacing with 2 stands of maize between 2 okra plants. The beneficiary effects of the cocoa pod husk as nutrient source was fully revealed when the marginal monetary value was calculated (Table 7). The marginal monetary values that resulted from additional unit tone of the cocoa pod husk were generally higher at 1.0 t ha^{-1}.

DISCUSSION

The sandy clay loam nature of the site soil coupled with the soil organic C content of 32 g kg^{-1} soil indicates that

Table 4. Maize grain yield under Okra/maize intercrop and CPH application.

Cropping pattern	Cocoa pod husk application rate (t ha^{-1})			
	0	1.0	2.0	4.0
	←	Maize grain yield (t ha^{-1})		→
2010 cropping				
Okra/Maize at 60 cm	3.40	3.70	4.38	3.56
Okra/Maize at 90 cm	4.03	4.53	5.27	4.81
Okra/Maize at 120 cm	4.46	4.81	5.37	5.09
Sole maize at 30 cm	4.63	5.46	5.55	5.67
LSD = 0.05	0.12	0.34	0.27	0.42
2011 cropping				
Okra/Maize at 60 cm	3.71	3.87	4.07	5.15
Okra/Maize at 90 cm	4.44	4.82	5.44	5.44
Okra/Maize at 120 cm	4.56	4.96	5.83	5.33
Sole maize at 30 cm	4.60	5.81	5.96	5.53
LSD = 0.05	0.35	0.21	0.15	0.31

LSD = Least significant difference.

Table 5. Land equivalent ratio (LER), land use saved and aggressivity in okra/maize intercrop.

Cropping pattern	Land equivalent ratio				Land use saved (%)				Aggressivity			
	Cocoa pod husk rate (t ha^{-1})				Cocoa pod husk rate (t ha^{-1})				Cocoa pod husk rate (t ha^{-1})			
	0	1.0	2.0	4.0	0	1.0	2.0	4.0	0	1.0	2.0	4.0
2010 cropping												
Okra/Maize at 60 cm	2.18	1.77	1.70	1.39	54.1	43.5	41.2	28.1	0.02	0.26	0.76	1.18
Okra/Maize at 90 cm	1.80	1.57	1.69	1.67	44.4	36.3	40.8	40.1	0.09	0.14	0.05	0.05
Okra/Maize at 120 cm	1.93	2.10	2.07	1.81	48.2	52.4	51.7	44.8	-0.65	-1.14	-0.74	-0.62
CV (%)	6.11	10.1	11.5	11.2	8.12	12.1	10.5	11.3	14.2	16.5	12.2	15.3
2011 cropping												
Okra/Maize at 60 cm	2.22	1.83	1.68	1.71	54.9	45.4	40.5	41.5	0.31	0.58	0.64	1.08
Okra/Maize at 90 cm	2.02	1.81	1.89	1.82	50.5	44.8	47.9	45.1	0.01	0.40	0.27	0.23
Okra/Maize at 120 cm	2.07	2.24	2.39	2.10	51.7	55.4	58.2	50.0	-0.67	-1.24	-1.08	-0.98
CV (%)	4.12	6.25	5.83	7.44	8.61	7.01	12.3	8.71	15.4	13.8	17.2	14.6

CV = Coefficient of variation.

the soil physical, biological and chemical conditions were moderate for optimum crop production (Singh et al., 2011). The soil pH was within pH 5.0 to 6.5 range which is considered adequate for arable crops (Crozier et al., 1999). However, the soil N, P and Ca contents were generally moderate, while the K and Mg contents were very low for either okra or maize cropping. This informed the need for application of nutrients for sustainable production of the crops, especially under intercropping systems, because, they are high nutrient demanding crops (Afolayan et al., 2006). The high N, P, K, Ca and Mg contents of cocoa pod husk, with C/N ratio of 16.6 showed that it could readily decompose, mineralize and

release nutrients in the soil for crop use (Ipinmoroti, 2013). Its high K and moderate Mg contents makes it a good nutrient source for soils that are low in K and Mg as depicted by the soil of the studied location.

Okra fruit yield was higher under okra/maize intercrop compared to values obtained under sole okra cropping except that the okra/maize intercrop was optimal at 90 × 75 cm okra spacing. The weight of okra fruits obtained increased with CPH application rate and the mean okra fruit yield differences were significant at p≤0.05. This suggests that better okra fruits could be obtained with proper nutrient management of the soil. This attests to the fact that the soil was inherently low in K and Mg

Table 6. Monetary value in naira (#000 t^{-1}) of okra and maize yield in okra/maize intercrop under cocoa pod husk (CPH) fertilizer treatment.

Cropping pattern	Crop	2010 cropping CPH rate (t/ha)				2011 cropping CPH rate (t/ha)				Total for 2010 and 2011 cropping CPH rate (t/ha)			
		0	1.0	2.0	4.0	0	1.0	2.0	4.0	0	1.0	2.0	4.0
Okra/Maize at 60 cm	Okra	333	476	507	527	278	446	434	492				
	Maize	1020	1110	1314	1068	1113	1161	1221	1545	2744	3193	3476	3632
	Total	1353	1586	1811	1695	1391	1607	1655	2037				
Okra/Maize at 90 cm	Okra	264	359	316	371	267	360	333	361				
	Maize	1209	1359	1581	1443	1332	1446	1632	1632	3108	3524	3862	3807
	Total	1473	1718	1897	1814	1599	1806	1965	1993				
Okra /Maize at 120 cm	Okra	130	433	400	380	131	410	388	365				
	Maize	1338	1443	1611	1527	1368	1488	1749	1599	2967	3774	4148	3871
	Total	1468	1876	2011	1907	1499	1898	2137	1964				
Okra sole 60 cm	Okra	229.8	435.3	575.3	693.3	201.9	435.1	477.5	632.5	431.7	873.4	1052.8	1324.8
Okra sole 90 cm	Okra	283.2	488.2	446.8	452.6	254.8	447.6	389.6	433.5	538.0	935.8	836.4	886.1
Okra sole at 120 cm	Okra	134.5	351.0	376.3	417.2	126.9	313.9	302.6	321.4	261.4	664.9	678.9	738.6
Maize sole	Maize	1387	1637	1604	1701	1320	1443	1579	1659	2707	3080	3183	3360

Table 7. Marginal monetary value in naira (₦000) in okra/maize intercrop for additional tone of cocoa pod husk (CPH) fertilizer application ha^{-1}.

Cropping pattern	Crop	2010 Cropping CPH rate (t/ha)				2011 Cropping CPH rate (t/ha)				Total for 2010 and 2011 cropping CPH rate (t/ha)			
		0	1.0	2.0	4.0	0	1.0	2.0	4.0	0	1.0	2.0	4.0
Okra/Maize at 60 cm	Okra	-	233	278	-58	-	216	48	191	-	449	283	78
Okra/Maize at 90 cm	Okra	-	245	179	-41.5	-	207	159	14	-	452	338	-27.5
Okra /Maize at 120 cm	Okra	-	408	135	-52	-	399	239	-86.5	-	807	374	-138.5

(Table 2), which are some of the major nutrient needs of the crop for optimum growth performance and better fruit bearing (Afolayan et al., 2006). The trend corroborates the reports of other authors that made use of organic fertilizers for maize, okra, tomato and vegetable production in Nigeria (Iyagba et al., 2012; Iremiren et al., 2007; Ayeni, 2010; Adeoye et al., 2001, 2005).

The better okra fresh fruit production under okra/maize intercrop compared to sole okra showed that the decomposition and eventual mineralization of the maize roots and the resultant corn stubbles must have improved on the soil organic matter, which must have subsequently helped to conserve the soil water, improved the physical, chemical and biological conditions of the soil (Fioretto et al., 2005). The maize stands in the intercrop must have helped to reduce impact of rain drops on the ground thereby improving water infiltration and water holding capacity of the soil, while the floor of the sole okra plot was left bare to heavy down pour of rain impact with its attendant side effects on the soil physical and chemical properties (Mbagwu and Obi, 2003).

The better performance of the CPH treated okra plants suggested that there is need for constant application of nutrients for sustainable okra cropping on the soil. This is true in that tropical soils could not support sustainable cropping over a long period without fertilizer application due to the fragile nature of the soils (Tolessa and Friesen, 2001). The general lower fresh okra fruit yield in 2011 compared to 2010 may probably be due to early stoppage of the rain in October, 2011 compared with late November in 2010 (Figure 1). Rainfall amount and distribution have been reported to affect cropping activities and eventual crop production in any particular cropping season, most especially fruit production (Omran, 2012).

The CPH application resulted to 4.49 to 23.53% increase in maize grain yield over the maize plants without CPH application. Maize grain yield under sole

cropping was however, higher than maize under okra/maize intercrop by 3.24 to 37.21% and 0.87 to 31.71% in 2010 and 2011 respectively. This was in similar trend with previous reports by Ijoyah et al. (2012), Iremiren et al. (2013, 2007) and Muoneke et al. (2007). The CPH application was optimum at 1.0 t/ha rate, while the mean differences between CPH application rates and cropping patterns for both cropping seasons were significant at p≤0.05. On the overall, the cropping pattern, fertilizer rate application and their interaction contributed significantly to the mean differences in yields of okra fruits and maize grain at both cropping seasons.

The land equivalent ratio (LER) values for okra and maize in the intercrop were greater than 1.0 with a range of 1.38 to 2.39 and about 28.1 to 58.2% of land area could be saved. The positive aggressivity values at 60×75 cm and 90×75 cm okra planting indicated that there was little or no deleterious competitiveness in the intercrop of the late maturing okra with maize. On the other hand, the negative values obtained at 120×75 cm okra planting showed that the maize component was too dense that both aerial and underground competition was too much (McGilchrist, 1965), that the okra plants could not express their full potentials in growth and fruit production. It would therefore not be advisable to ask farmers to adopt such a planting pattern for the late maturing okra in okra/maize intercrop for sustainable farming business. Nigerian farmers are used to polyculture practice, especially in respect to arable farming (Okpara and Omaliko, 1995), hence, the best of the okra/maize intercropping patterns with productivity advantage on LER and aggressivity would therefore be advisable to the farmers.

The okra/maize intercrop resulted to significantly (p≤0.05) higher monetary values (MV) compared to either okra or maize cultivated sole. The monetary values were generally highest at the 120×75 cm okra spacing in the intercrop. However, the cropping pattern could not be recommended to farmers because of the negative aggressivity values and the attendant implications that would result from competitions between the component crops in the intercrop (Iyagba et al., 2012; McGilchrist, 1965). The monetary value was higher at 90×75 cm than 60×75 cm okra spacing in the intercrop, with positive aggressivity. Therefore, putting all the productivity indices into consideration, the 90×75 cm okra spacing in okra/maize intercrop becomes the most optimal cropping pattern.

The combined monetary values for 2010 and 2011 cropping seasons showed the superiority of okra + maize in okra/maize intercrop over sole okra or maize cropping. The okra/maize intercrop was optimal at the 90×75 cm okra spacing with 2 stands of maize between 2 okra plants. The marginal monetary values that resulted from the use of additional unit tone of cocoa pod husk as nutrient source was generally higher at application rate of 1.0 t ha^{-1}. Hence, cocoa pod husk application at 1.0 t ha^{-1} was found to be more economical.

Conclusion

Cocoa pod husk resulted in significant increased yield of okra and maize plants with increased rate of application than control without fertilizer. Okra and maize yields were optimum at okra/maize intercrop planted at 0.90×0.75 m with 2 maize stands between 2 okra stands with cocoa pod husk application at 1.0 t ha^{-1}. The land equivalent ratio (LER) was greater than 1.0 across the various cropping patterns of okra/maize intercrop. However, the aggressivity values were negative when okra was planted at 1.20×0.75 m with 3 maize stands between 2 okra stands, which indicates serious competition between the component crops. The monetary value (MV) was more under okra/maize intercrop but the marginal monetary value (MMV) of CPH application was optimum at 1.0 t ha^{-1} when okra was planted at 0.90×0.75 m with 2 maize stands between 2 okra stands and was recommended.

Conflict of Interest

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

REFERENCES

Adeniji OT (2007). Genetic studies on earliness in West African okra (Abelmoschus ceillei [A. chev.] Stevels). Int. J. Agric. Res. 2:750-753. http://dx.doi.org/10.3923/ijar.2007.750.753

Adeoye GO, Sridhar MKC, Adeoluwa OO, Akinsoji NA (2005). Evaluation of naturally decomposed solid wastes from municipal dumpsites for their manurial value in southwest Nigeria, J. Sustain. Agric. 26(4):143-162. http://dx.doi.org/10.1300/J064v26n04_09

Adeoye GO, Sridhar MKC, Ipinmoroti RR (2001). Potassium recovery from farm wastes for crop growth. Communications in Soils and Plant Anal. 32(15-16):2347-2358. http://dx.doi.org/10.1081/CSS-120000377

Afolayan SO, Bindrabam P, Ajayi E (2006). Performance evaluation of drip irrigation and fertigation systems on Telfairia occidentalis in Ibadan, south western Nigeria. Nig. J. Hortic. Sci. 11:36-41.

Agbede OO, Kalu BA (1995). Constraints of small-scale farmers in increasing crop yields: Farm size and fertilizer supply. Nig. J. Soil Sci. 11:139-152.

Ayeni LS (2010). Effect of cocoa pod ash, NPK fertilizers and their combinations on soil chemical properties and yield of tomato (Lycopersicom lycopersicom) on two soil types. New York Sci. J. 3(4):1-11.

Bamire AS, Oke JT (2003). Profitability of vegetable farming under raining and dry season production in south-western Nig. J. Veg. Crop Prod, 9:11-18. http://dx.doi.org/10.1300/J068v09n02_03

Bray RA, Kurtz LT (1945). Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59(3):9-45.

Bremmer JM (1996). Methods of Soil Analysis, Part 3: Soil Science of America and America Society of Agronomy SSSA Book series No. 5, Madison, USA.

Crozier CR, Naderman CC, Tuckan MR, Sugg RE (1999). Nutrient and pH stratification with conventional and no-till management. Communications in Soil Sci. Plant Anal. 30(1&2):65-74. http://dx.doi.org/10.1080/00103629909370184

Farinde AJ, Owolarafe OK, Ogungbemi OI (2007). An overview of production, processing, marketing and utilization of okra in Egbede Local Government Area of Osun State, Nig. Agric. Eng. Int. 9:1-17.

Fioretto AC, Nardo D, Papa S, Fuggi A (2005). Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biol. Biochem. 37:1083-1091. http://dx.doi.org/10.1016/j.soilbio.2004.11.007

Gomez AA, Gomez KA (1983). Multiple cropping in the humid tropics of Asia. IDRC, Ottawa. IDRC-Abe. P. 249.

Ijoyah MO, Adagba EO, Iorlaman T (2012). Productivity of okra-maize intercropping system as influenced by varying maize plant densities in Makurdi, Nig. Int. J. Curr. Res. 4(4):059-063.

Iken JE, Amusa NA (2004). Maize research and production in Nigeria. Afr. J. Biotechnol. 3:302-307.

Ipinmoroti RR (2013). Decomposition and nutrient release patterns of some farm wastes under control room temperature. International J. Agric. Forest. 3(4):185-189.

Iremiren GO, Okiy AO (1999). Effect of spacing on the growth, yield and quality of okra in southern Nig. J. Agric. Sci. 10(1):12-26.

Iremiren GO, Ipinmoroti RR, Hamzat RA, Adedeji RA (2007). Effect of fermentation period of kola pod husk, Time of application and urea supplementation on growth, yield and sensory properties of okra (*Abelmoschus esculentus* L). J. Appl. Trop. Agric. 12(2):70-72.

Iremiren GO, Ipinmoroti RR, Akanbi OSO (2013). Performance of Okra (*Abelmoschus esculentus* L.) and Maize (*Zea mays*) under Okra/Maize intercrop as influenced by nutrient sources at Ibadan, Nig. Int. J. Plant. Soil Sci. 2(2):190-201. http://dx.doi.org/10.9734/IJPSS/2013/4796

Iyagba AG, Onuegbu BA, Ibe AE (2012). Growth and yield response of okra (*Albelmoschus esculentus* [L.] Moench) varieties to weed interference in south eastern Nigeria, Global J. Sci. Front. Res. Agric. Veter. Sci. 12(7):1-17.

McGilchrist CA (1965). Analysis of competition experiments. Biometrics, 21:975-985. http://dx.doi.org/10.2307/2528258

Mead R, Wiley RW (1980). The concept of a land equivalent ratio and advantages in yields from intercrop. Exper. Agric. 16(3):217-228. http://dx.doi.org/10.1017/S0014479700010978

Muoneke CO, Ogwuche MAO, Kalu BA (2007). Effect of maize planting density on the performance of maize/soy bean intercropping system in a Guinea savanna agroecosystem. Afr. J. Agric. Res. 2(12):667-677.

Nelson DW, Sommers LE (1982). Organic carbon and soil extracts. In: Methods of soil Analysis, Part 2,- Chemical and microbiological properties. Agronomy Monograph No. 9, 2nd Edition, American Society of Agronomy, Soil Sci. Soc. Am. Madison, WI, USA. pp. 539-579.

Mbagwu JSC, Obi ME (2003). Land degradation, agricultural productivity and rural poverty: Environmental implication. Proceedings of the 28th Annual Conference of the Soil Science Society of Nigeria, held at Umudike, Umuahia, Nigeria, 4-7 November, 2003; pp. 1-11.

Okpara DA, Omaliko CPE (1995). Productivity of yam in bean/yam intercropping. Indian J. Agric. Sci. 65(12):880-882.

Omran WM (2012). Quantifying Medicago sativa yield under deficit irrigation technique in sandy soil. Int. J. Plant. Soil Sci. 2(2):202-211. http://dx.doi.org/10.9734/IJPSS/2013/4981

Raji JA (2007). Intercropping soy bean and maize in a derived savanna ecology. Afr. J. Biotechnol. 6(16):1885-1887.

Singh CM, Shamarma PK, Kishor PK, Mishra PK, Singh AP, Verma R, Raha P (2011). Impact of integrated nutrient management on growth, yield and nutrient uptake of wheat (*Triticum aestivum* L.). Asian J. Agric. Res. 5(1):76-83. http://dx.doi.org/10.3923/ajar.2011.76.82

Tel D, Rao F (1982). Animated and semi-animated methods for soil and plant analysis pp. 201-207.

Tolessa D, Friesen DK (2001). Effects of enriching farmyard manure with mineral fertilizers on grain yield maize at Bako western Ethiopia. Seventh Eastern and Southern Africa Regional Maize Confer. pp. 335-337.

Uzozie PR (2001). Effect of intercropping maize on the performance of cassava-maize intercrop. J. Agric. Sci. 12:18-21.

Yadev SK, Dhanker BS (2002). Performance of 'Varsha Uphar' cultivar of okra as affected by growing dates and plant geometry. Veg. Sci. 27:70-74.

Full Length Research Paper

Market trend in the agribusiness sector of hybrid maize seed in Paraná State, Brazil

Leandro Bonadio Machado[1], Valéria Carpentieri-Pípolo[1,2]* and Silvia Graciele Hülse de Souza[3]

[1]Post Graduation Program in Agronomy , Universidade Estadual de Londrina, CP 6001, Londrina, PR, 86051-990, Brazil;
[2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Trigo, Rod BR 285, Km 294, CP 451, Passo Fundo-RS, 99001-970, Brazil.
[3]Laboratory of Molecular Biology, Universidade Paranaense, CP224, Umuarama, 87502-210, Brazil.

Nowadays maize hybrid seed companies use marketing tools to achieve their goals on the target markets in order to stand up to competition, meet consumers' demands, and ensure the market share. The objective of this study was to identify and rank the variables consumers consider most in the product purchase decision and to survey technical and behavioral aspects to create actions and strategies for the companies of the sector. For this purpose, surveys were conducted with producers of the region of Mauá da Serra (Paraná State) based on the four Ps of the marketing mix (product, promotion, price, and place). In the product mix the factors grain yield, performance in the previous year and recommendations of research institutions influence consumers most in the buying decision. Financing is the preferred form of payment for seeds, and special offers have more influence on the buying decision than the price itself. The months of May, June and July are preferred for seed purchase. The consumers use different media channels to obtain information about the product but what influences producers most are lectures and field days. Companies that apply marketing strategies ensure competitiveness and consumer fidelity.

Key words: Aimed market, marketing-mix, seed companies, strategies, *Zea mays*.

INTRODUCTION

Today, Brazil is the third-greatest maize producer, after the Unites States and China. In the 2013/2014 growing season, maize was planted on 15.01 million hectares with a total production of 75.465 million tons of grains (32.636 in the 1st and 42.829 in the 2nd growing season, respectively). Paraná produced 15.775 million tons of grains (5.624 in the 1st and 10.151 in the second 2nd growing season), which corresponds to 20.9% of the Brazilian production, confirming the leading position of the state as largest producer of maize in Brazil (CONAB, 2014). The interest for high technology seeds, namely in the market of hybrid seed, is on the rise. In the growing season 2013/2014, there was a predominance of simple hybrids (56.15%). The triple hybrid (18.61%), double hybrids (13.56%) and variety (11.68%) complement the options market (EMBRAPA, 2014). Since the growing
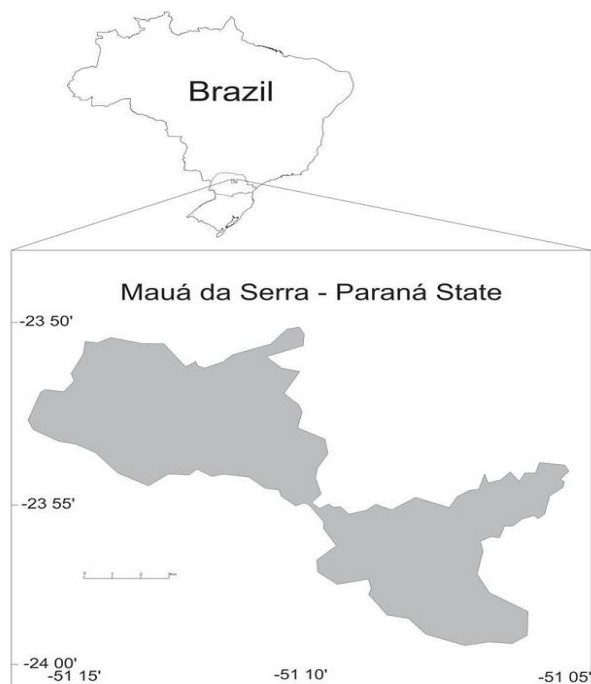
Figure 1. Area covered in the sample is marked in gray.

season 2005, there has been an increasing in the availability of simple hybrids on the market. In recent years, this type of cultivar is more than 50% of the available seeds (Cruz et al., 2014). This expresses a trend in the Brazilian agriculture and a growing demand for enhanced production systems.

Seed companies in Brazil have attained an excellent level of technological development in the field of seed production. The increased use of improved seeds resulted in the growth and diversification of seed production, provided mainly by the evolution of plant breeding, the use of biotechnology and the incorporation of new technologies to the seed production process (Foedermayr and Diamantopoulos, 2008). However, only this factor is no longer a competitive differential that would ensure a company's competitiveness and survival on the market (Spielman et al., 2014). Companies must therefore be capable of identifying their advantages and disadvantages. For this purpose, specific activities that give a competitive edge need to be identified (Mawehe, 2008). Competitors must be different enough to have at least one unique advantage. This virtue can be expressed in a different price of sale, functions, localization, or even in the idea the client has of the product and the distributor (Porter, 1996; Henderson, 1998). In this situation, market research based on marketing strategies is necessary for any company and seed companies are no exception (Anurag and Priyanka, 2012). Girardi (2002) evaluated a marketing composite in the agribusiness of soybean seeds in the state of Paraná, and observed the need to differentiate existing brands in

the market. Making seeds available soon after harvest and deferred terms of payment can stimulate the interest of purchase. Events such as field days and technical assistance and the use of referential producers were the most efficient forms of promoting the product.

"Marketing mix" is a set of tools that a company applies to achieve marketing objectives on a target market (Kotler, 1998; Feeney and Berardi, 2013). There are countless such tools, but McCarthy (1978) proposed a classification of these tools that became popular in marketing science, the so-called four Ps (product, price, place, and promotion). On this background, our study aimed to identify the factors that most influence farmers in the choice and acquisition of maize seeds, with a view to strategic changes in the areas of trade and production by planning effective actions in cooperation with the clients.

MATERIALS AND METHODS

This study was carried out together with farmers in the region of Mauá da Serra – Northern Paraná State, Brazil (Figure 1). At present, the cooperative has 96 members and represents a regionally important center of technology dissemination. Mauá da Serra is state and nationwide outstanding in view of consecutive yield records in maize production.

A survey proposed by Girardi (2002) was used as an instrument of evaluation and diagnosis. It consists of qualitative questions that determine the client's profile and questions related to the 4 Ps of marketing mix (product, price, place and promotion) to diagnose and define the market strategy for the propagation of the product to improve client satisfaction.

A sampling system was used to define how many producers should be interviewed to establish an accurate result. The sample size was determined by a formula proposed by Richardson (1999):

$$n = \sigma^2.p.q.N/ E^2. (N-1) + \sigma^2 p.q$$

Where: n = sample size; σ^2 = determined confidence level, in number of deviations (sigmas); p = proportion of the surveyed feature in the whole-sample, in percentage; q = 100 − p (proportion of the whole-sample that does not have the surveyed feature, in percentage); N = population size; E^2 = allowable error of estimation.

In the particular situation of this study: n = is the intention; σ^2= 22; p = 50%; q = 100 − 50% = 50%; N = 96; E^2 = 5%^{2}. A sample size of 77.57 was calculated - in other words, 78 producers answered the survey.

The data were organized and analyzed using descriptive statistical methods, as proposed by Giradi (2002). The analysis had the objective of organizing the data in a way that would help find answers to the problem proposed in the investigation.

RESULTS AND DISCUSSION

The producers' profile

The maize producers in the study group were young;

80% were 20 to 40 years old (30% were 21 to 30 and 50% 31 to 40 years old) and the other 20% were between 50 and 60 years old. Sixty percent of the farmers interviewed had completed a college degree. To the question about their experience in maize cultivation, 40% of the producers answered that they have been growing maize for over 21 years, 30% between 10 and 20 years and 30% between two and six years.

The high educational level of these relatively young farmers can explain their interest in keeping up with technological development. One hundred percent of the producers plant early and semi-early hybrids, use treated seeds and 95% have implanted the no-till cultivation system. When asked about the quality of information and the type of divulgation there was a preference for lectures, which can also be associated to the educational level of the interviewees. Forty percent of the producers grow maize on an area of up to 199 ha, 10% over 600 ha and 40% from 200 to 600 ha. Sixty percent of the interviewees produce an average 8000 to 10000 kgha^{-1} of maize, 30% produce 5000 to 8000 kg ha-1 and 10% over 10,000 kg ha^{-1}.

Product

In the growing season 2013/2014, 467 maize cultivars were released of which 422 (90.37%) cultivars are released by private companies (EMBRAPA, 2014). This may be reflection of technological partnerships between breeders, mainly holders' biotechnology, private seed companies and public institutions and/or universities. This choice business has become common in Brazil, whose aim is always pursue specific solutions, aimed at obtaining a differentiated product. This modality tends to grow worldwide, since most companies do not have all the technical and scientific expertise and financial capacity required for the development all stages of the creation of a new product.

The seed product can be defined as a complex of tangible and intangible attributes that can, when offered on the market, satisfy the desire or need of the farmer clients (Acosta et al., 2002a). When producing commodities or related products, a larger number of clients can be attracted by a differentiation of the final product and aggregation of values and association of services that make the product more attractive and competitive than the competitor's (Wilkinson, 1995). One of the strategies adopted by companies whose competitors offer the same benefits, product quality and price, is to provide a product of differentiated packaging, of appealing appearance and convenient handling. In relation to the seed product, 100% of the producers prefer cardboard wrapping (Table 1). The farmers' preference for cardboard packaging must be associated to the ease of use and convenience this wrapping provides; besides, it offers greater safety in the manipulation of these frequently fungicide-treated seeds on the field besides being readily disposable and environment-friendly.

With regard to the seed quality - in contrast to the study of Peske et al. (1991), who concluded that seeds were chosen and bought mainly for a high germination rate - in this study the farmers cited vigor most often (26%) (Figure 2) as responsible for quality. Germination and seed size ranked second with 17%, followed by the developing company, variety mixture and origin with 10% each. Half of the interviewees stated that there are problems with germination and low vigor of the seed available on the market.

To purchase seed for planting, 50% of the interviewed farmers did not relate the choice of the seed to the brand. The majority (70%) considered the performance of the variety in the previous year as an influence on the seed choice, as well as yield (95%), seed size (60%) and cycle (90%). They preferred early and semi-early varieties and fungicide- treated seeds (70%) and 100% of the interviewees considered resistance to pests and diseases fundamental for choosing a cultivar. The increase of the area where maize is planted in the 2nd growing season has increased the demand for traits such as earliness and disease resistance.

Of the services provided, recommendations of the technical assistance staff also have an expressive mean influence (65%) (Figure 3) on the choice of the cultivar, compared with the recommendation of research institutions (80%). Half of the farmers interviewed were unsatisfied with the technical assistance provided by the companies. They describe a lack of adequate technical information about seeds, besides disaffection with the reception of customer complaints. The use of these strategies of orientation and service to farmers is still far from ideal and must be improved if the companies of this sector are to obtain customer satisfaction and fidelization.

Price

Seeds can be considered one of the most important investments in the production systems that involve commercial crops (Jaffee and Srivastava, 1992). More than just a mere raw material, seed is a carrier of the technology of the cultivar, a highly effective technologic vector, so this technology used in the seed development is included in the price (Acosta et al., 2002b).

With respect to the seed price charged by the companies, 80% of the farmers claim that prices are high. Maize prices hit average of the last three harvests the amount of $9.0 per kilo of seed simple hybrid (ABRATES, 2014). These farmers mainly take the price of commercial sacks/ha and the ratio sacks of grain to sacks of seed as basis for the price comparison of maize seed. One of the price strategies adopted by companies is the practice of a competitive price, that is, the product is sold at a lower

Table 1. Degree of agreement according to the marketing composite in percentage.

Product	(1)	(2)	(3)
The trademark is a relevant factor when buying seed.	40	50	10
There are constantly problems with maize seed germination.	38	56	6
Yield is not an important factor when choosing the maize cultivar.	0	5	95
There are no problems with low vigor in maize seed.	0	45	55
The cycle is an important factor in the choice of the maize cultivar.	90	10	0
There is not enough adequate technical information on maize seed.	20	50	30
The performance in the previous year is not relevant when choosing the maize cultivar to be planted.	10	20	70
Technical assistance staff assigned to maize plantations does not seem well prepared.	30	50	20
Seed size has no influence on which maize cultivar is chosen.	10	50	40
There are no problems of variety mixture in maize seed.	40	50	10
Maize seed must be fungicide-treated.	90	10	0
The response to customer complaints is effective.	20	30	50
Research-based recommendations (Embrapa, IAPAR, others) have an influence on the choice of the maize cultivar to be planted.	80	15	5
Resistance to pests and diseases influences the choice of the maize cultivar.	100	0	0
Recommendations of the technical assistance staff do not influence the choice of the maize cultivar to be planted.	20	15	65

Price			
The price is an important factor at buying maize seed.	50	40	10
The price of maize seed is not high.	0	20	80
Discounts are important factors at the moment of purchase of maize seed.	80	20	0

Point of distribution			
The service of technical assistance is not very important when deciding on the point of sale of maize seed.	0	30	70
Maize seed is delivered within the specified period.	40	60	0
The maize cultivars ordered are not the ones that are actually delivered.	0	45	55
Maize seed must be delivered to the doorstep.	50	30	20

Promotion			
Maize seed vendors are generally ill-prepared professionals.	30	40	30
The material sales campaigns do not seem to be a good opportunity for buying maize seed.	40	50	10
The performance of other producers has no influence on the choice of the maize cultivar.	0	50	50
Advertising in radio, television, newspaper, leaflets or other material of publicity do not seem to affect the choice of the maize seed cultivar for planting.	70	30	0
Lectures have an influence on which maize cultivar is chosen.	80	20	0
Observations on field days have no influence on the choice of the maize cultivar.	0	20	80

(1) Agree, (2) agree partially and (3) disagree.
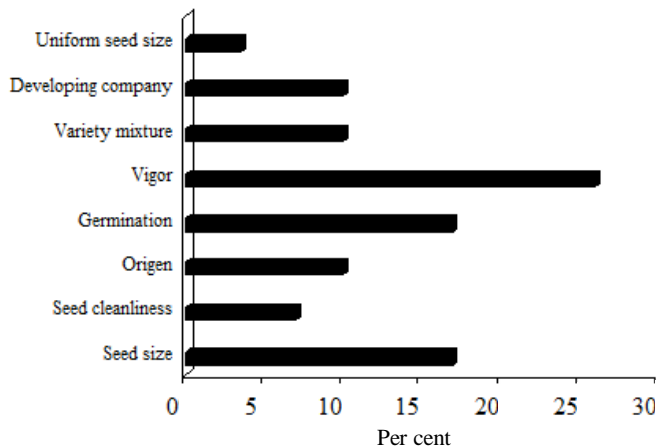
Figure 2. Technical characteristics in maize seed producers consider important.
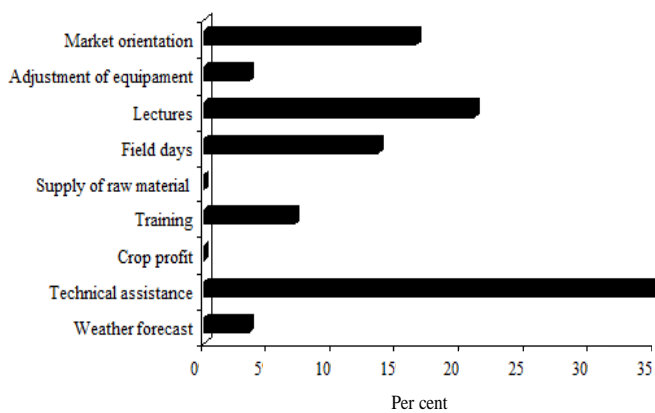
Figure 3. Services the producers think a maize seed distribution company ought to provide.

price than the competitor's. This strategy is normally successful when products are basically the same and achieve a high sales volume at low costs. An adoption of this strategy by seed companies is not always recommendable since only 50% of the farmers consider the price in the seed choice whereas 80% of the interviewed assume a sales discount as relevant. This can be used by the companies as a great attraction to make seed purchase appealing for clients.

With regard to the form of payment, 78% of the sample group prefers to pay the seeds on an installment plan versus only 22% who prefer a single payment. In Brazil, rural credit is provided by credit cooperatives and by the commercial banks. In general, the interest rate charged on the loans ranges from 1% to 14% per annum and the payment term varies according to the funding source, the purpose and the production plan submitted. In spite of boosting sales, this is an expensive strategy for the

retailer who has a large quantity of outstanding payments, while the opportunity of reinvestment in any other investment type is lost. For the producer on the other hand, the forms of payment, as well the lack of term of repayment and high interest rates can complicate and even cause the abandonment of the purchase process (Teixeira et al., 2004). Maize seed clients are concerned about the price, although it is not the central factor, since the form of payment and the discounts play an important role as well.

Point of distribution

Distribution is the movement of the product from the point of production to the final consumer and is related with distribution channels, number of sales outlets, range of products, location, stock and transport. Cooperatives sell 60% of all seed, compared with other stores. Seed purchase is most concentrated in the months of May, June and July (20%, 40% and 13%, respectively). This information is useful for companies since seed sales campaigns can be intensified in this period. Seventy percent of the interviewed farmers mention that the technical assistance staff has a great influence on the choice of the place of purchase. Sixty percent of farmers interviewed consider that the maize seed is delivered within the specified period. Moreover, almost 50% of the farmers state that the companies are not prepared to attend purchase orders properly; in 45% of the cases they buy a certain variety but receive another, demonstrating disaffection of the producer with this service.

Promotion

The promotion of a product is an important component for setting up and developing the promotional compound and can be defined as the process of company-client communication (Acosta et al., 2002a). Promotion strategies are determined according to a company's objectives and the most adequate tools to achieve these objectives are chosen. As much as on the product itself, the focus must be on the communication process, namely advertisement, sales promotion, publicity and personal sales (Boas et al., 2004).

In relation to the promotion of the product, 32% of the interviewees have unrestricted access to newspapers, 28% to TV, 22% to magazines and 13% to internet. According to Dias (1993), advertisement is one of the strongest weapons to stimulate the product-market link, since it is one of the main factors for formation of the product sales volume at the point of sales. One must however take some aspects into account: the outreach of an advertisement, that is, the percentage of the target market that is influenced by advertising; the frequency,
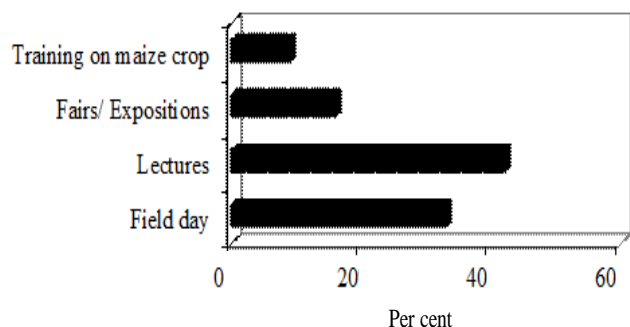
Figure 4. Events about maize producers attend to.

that is, the number of times a person is reached by the message; and the appeal of the message - it must spark interest in the product, creating the desire and finally the action of buying. This kind of promotion is therefore an area seed companies can also invest in to increase sales.

Among the producers interviewed 36% take information of technical assistants into consideration while only 18% consider the opinion of researchers for the seed choice. Only 14% of the farmers consider the opinion of seed sellers and another 14% the opinion of other producers. For the seed choice the interviewed obtained information on the cultivars in equal shares from technical bulletins, technical magazines and informative leaflets (33%). The participation of maize producers in technical events is quite high (72%) and can be further exploited according to the potential of preference, mainly lectures (40%) and field days (32%) (Figure 4). Eighty percent of the interviewees agreed that field days and lectures have an influence on the seed choice. According to Peske et al. (1991), these types of events were the main means used to promote cultivars and the company's image.

Conclusion

The surveyed farmer is demanding with respect to customer liaison and support. Based on this observation, technical assistance is a strong strategy to achieve and consolidate a market share and form a public opinion about the company trademark. Besides representing a diagnostic instrument for the inclusion of new products and services, the technical assistance staff is assigned to explain the producers that the all-in price includes the technology required for the different production systems, in each growing season. Brazil has two seasons for maize production. First-crop maize is planted in September and is harvested in March. Production largely serves the domestic feed market. Second-crop corn is planted in January after the early-season soybean harvest. Production from the second corn crop is destined for the export market. Considering that among

the interviewees, the main way of seed purchase is through the cooperative and since farmers prioritize an individual customer service, technical assistance may be the most powerful tool of sales boosting and product publicity. This is however only possible, if the cooperative invests in the qualification and training of the technical staff and provides the client with all technical support of the differentiated high quality service he considers important and differential for seed purchase.

A company can find out what clients really appreciate and try to find means to understand and provide these "values". Normally, technical characteristics are differentials if the product is exclusive in a point esteemed by the client. Services rendered, together with the product, usually give rise to the so-called amplified product. We can find a number of forms of establishing an esteemed differential through these services, in other words, a real differential.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

Acosta A, Barros ACSA, Peske ST (2002a). Diagnóstico setorial aplicado às empresas de sementes de trigo e soja do Rio Grande do Sul. Rev. Bras. Sementes 24:71-80.

Acosta A, Barros ACSA, Peske ST (2002b). Tipologias e composto mercadológico de empresas de sementes de trigo e soja do Rio Grande do Sul. Rev. Bras. Sementes 24:81-90.

Anurag SB, Priyanka T (2012). Marketing strategies for rural market. South Asian J. Mark. Manage. Res. 2(9):80-104.

ABRATES (Associação Brasileira de Tecnologia de Sementes) (2014). Available at: http://www.abrates.org.br. Accessed 12 Sep. 2014.

Boas LHBV, Antonialli LM, Sette RS, Mario TMC, Luna RM (2004). Marketing dos agentes do agronegócio do café: Uma análise sob a ótica do marketing mix. Revista de Administração da UFLA 6:92-106.

CONAB (Companhia Nacional do Abastecimento) (2014). Available at: http://www.conab.gov.br. Accessed 02 Mar, 2014.

Cruz CC, Pereira Filho IA, Silva GH (2014). Milho: Cultivares para 2013/2014. Available at: http://www.cnpms.embrapa.br/milho/cultivares/index.php. Accessed 20 Sep. 2014.

Dias SR (1993). Estratégia e canais de distribuição. São Paulo: Atlas, 368 p.

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) (2014). Available at: http://www.cnpms.embrapa.br/milho/cultivares. Accessed 08 Mar. 2014.

Feeney R, Berardi V (2013). Seed Market Segmentation: How Do Argentine Farmers Buy Their Expendable Inputs? Int. Food Agribus. Man. 16(1):17, 40.

Foedermayr E, Diamantopoulos A (2008). Market Segmentation in Practice: Review of Empirical Studies, Methodological Assessment, and Agenda for Future Research. J. Strat. Market. 16(3):223-265. http://dx.doi.org/10.1080/09652540802117140

Girardi RE (2002). Estratégias de marketing no agronegócio de semente de soja. Dissertação de Mestrado, Programa de Pós-Graduação em Engenharia de Produção, Universidade Federal de Santa Catarina, Florianópolis, 124 p.

Henderson BD (1998). As origens da estratégia. In: Montgomery C, Porter M (Org.) Estratégia: a busca da vantagem competitiva. Rio de Janeiro: Campus pp. 3-9.

Jaffee S, Srivastava J (1992). Seed system development: the

appropriate role of the private and public sectors. Washington: World Bank, 69 P. (World Bank. Discussion Papers, 167).

Kotler P (1998). Administração de marketing: análise, planejamento, implementação e controle, 5ed São Paulo: Atlas, P. 728.

Mawehe J (2008). Agricultural marketing information and maize marketing in Zambia. A Masters Dissertation in Business Administration, The Copperbelt University, Kitwe, P. 94.

Mccarthy EJ (1978). Marketing básico: Uma visão gerencial. 2ed. Rio de Janeiro: Zahar, 2:1302.

Peske ST, Baudet L, Barros AC, Irigon DL (1991). Perfil do agricultor em relação ao uso de sementes, Inf. Abrates 1:47.

Porter M (1996). What is strategy? Harvard Business Review, pp. 61-78.

Richardson JR (1999). Pesquisa social: Métodos e técnicas. São Paulo: Atlas, P. 334.

Spielman DJ, Kolady DE, Cavalieri A, Rao NC (2014). The seed and agricultural biotechnology industries in India: An analysis of industry structure, competition, and policy options. Food Policy 14:88-100. http://dx.doi.org/10.1016/j.foodpol.2014.01.001

Teixeira L, Neves MF, Scare RF (2004). Auditoria e Sistema de Informação em Canais de Insumos do Agronegócio: Proposta de uma ferramenta pesquisa. Gestão Produção 11:399-411. http://dx.doi.org/10.1590/S0104-530X2004000300012

Wilkinson J (1995). Competitividade da agroindústria brasileira. Agric. em São Paulo 42:27-56.

academicJournals

African Journal of Agricultural Research

Full Length Research Paper

Biological and serological techniques for detection of *Citrus tristeza* virus affecting *Citrus* species of Assam, India

M. Borah*, P. D. Nath and A. K. Saikia

Department of Plant Pathology, Assam Agricultural University, Jorhat 785013, Assam, India.

Infection of Citrus tristeza virus (CTV) in Assam was detected by double antibody sandwich-enzyme-linked immuno-sorbent assay (DAS-ELISA). Field surveys were carried out in 8 citrus growing districts of Assam (Tinsukia, Lakhimpur, Jorhat, Kokrajhar, North Cachar Hills, Karbi Anglong, Golaghat and Kamrup). Altogether, 411 samples were collected from three different citrus species, viz., Assam lemon (*Citrus limon*), Gul nemu (*Citrus jambhiri*) and Khasi mandarin (*Citrus reticulata*) to test against CTV. Results of biological indexing showed Kagzi lime (*Citrus aurantifolia*) to be the better indicator plant (symptom expression 60%) than Assam lemon (symptom expression 30%). The symptom expression on the indicator host was observed from 5th to 9th week after inoculation. The inoculated plants again tested after 9th week using DAS-ELISA assay. The results revealed that percentage of positive plants were 90% in Kagzi lime followed by Assam lemon (80%). This indicated that even the symptomless plants after inoculation give the positive reactions in DAS- ELISA assay. 261 samples were found to be infected using ELISA with polyclonal antisera to CTV. The species Assam lemon (*C. limon*) were found to be susceptible to CTV with estimated disease incidence up to 76.47% followed by Khasi mandarin (*C. reticulata*; 61.18%) and Gul nemu (*C. jambhiri*; 52.03%). Different age groups (<10, 10 to 15 and >15 years) of the citrus trees indicated that prevalence of CTV was more in older plants.

Key words: ELISA, Citrus tristeza virus (CTV), Assam lemon, Khasi mandarin, Gul nemu.

INTRODUCTION

Citrus is considered to be one of the most remunerative fruit crops of India, having a lasting niche in the international trade and world finance. Most of the *Citrus* species are believed to be native to tropical and subtropical regions of Southeast Asia, particularly Northeastern India and the region between China and India (Ghosh, 2007). The Northeastern region of India is considered as the home of mandarin orange and many citrus fruits (Ghosh, 2007). Sub mountain and hilly tracts of states of Northeast, Assam, Meghalaya, Manipur, Arunachal Pradesh, Mizoram, Nagaland, Tripura, Sikkim and the Darjeeling hills of West Bengal grows excellent quality citrus fruits like, mandarin (Darjeeling, Khasi and Sikkim mandarin), sweet orange, lemons and limes. Khasi mandarin is the most important commercial cultivar of Northeast India, followed by Assam lemon, which is very popular in homestead gardens. Apart from these two cultivars, rough lemon (*Citrus jambhiri* Lush.), citron

(*Citrus medica* L.), and sweet orange (*Citrus sinensis* Osbeck) are also grown in this region (Verma and Ghosh, 1979).

The global problem of citrus decline warranted special attention of agricultural scientists during the past few decades. The tristeza virus and viroid exocortis greening is caused by a fastidious bacterium in association with some fungi, nematodes and bacteria have been found to be involved in the citrus die back complex in India. Among the seven aphid species reported as vectors of *Citrus tristeza* virus (CTV), *Toxoptera citricida* has been found to be most efficient vector (Capoor and Rao, 1967). CTV is a phloem limited 2000 nm long filamentous virus having single stranded RNA genome (Karasev et al., 1995). The virus is genetically and biologically diverse and can cause field symptoms ranging from vein clearing, stem pitting, yellowing, slow decline and quick decline, or no symptoms depending on virus isolate, time of infection, root stocks, citrus cultivars and environmental conditions (Bar-Joseph et al., 1989).

Extremely limited information and experimental data are available on the occurrence and spread of CTV in the Northeastern region of India. However, a survey and indexing results recorded presence of tristeza in Khasi orange, Kagzi lime and Assam lemon in the Northeastern region in India (Bhagabati et al., 1989). The biological-indexing is relatively less reliable and time consuming, but double antibody sandwich-enzyme-linked immuno-sorbent assay (DAS-ELISA) has revolutionized the detection test, making it feasible to test large number of samples (Bar-Joseph et al., 1979; Cambra et al., 1979; Chakraborty and Ahlawat, 2001). In recent years, PCR based diagnosis has been used for the rapid diagnosis of citrus virus (Biswas, 2010; Edson et al., 2008). ELISA test can provide rapid, sensitive and economical detection of CTV in crude extracts from citrus trees.

The viral infection in plants alters the metabolism and physiology of the plant; hence, it is necessary to conduct an indexing of the viral disease to analyze the percentage of infection to control the disease in future. Diagnosis based on visible symptoms is generally unreliable and thus advanced diagnostic tools like ELISA and RT-PCR that has been used earlier in India (Chakraborty et al., 1992; Biswas, 2008), are very much essential for detection of CTV accurately. In the present study, advanced diagnostic tool that is, ELISA have been employed for detection of CTV. A comparative study on traditional method of diagnosis (that is, biological indexing) and antibody based detection technique (that is, DAS-ELISA) have been conducted and evaluated their efficiency.

MATERIALS AND METHODS

Survey of *Citrus tristeza* virus (CTV) infection and aphid population

Survey and investigation was conducted in four agroclimatic zones viz., Upper Brahmaputra Valley zone, Lower Brahmaputra Valley zone, North Bank Plain zone and Hills zone of Assam covering eight districts (Jorhat, Tinsukia, Golaghat, Lakhimpur, Karbi Anglong, North Cachar Hills, Kamrup and Kokrajhar) during 2009-2010. Three to five locations from each district were surveyed for CTV infected plants. Suspected CTV infected samples from Khasi mandarin (*Citrus reticulata*), Gul nemu (*C. jambhiri*) and Assam lemon (*Citrus limon*) were collected from both commercial and home stead gardens of citrus trees from the age group of <10 years, 10 to 15 years and above 15 years trees. Altogether, 411 leaf tissue samples were collected from three different citrus species (Table 1).

Although symptoms of greening were also observed in all the surveyed locations on the investigation primarily concentrated on the CTV infection. There may be a mixed infection of greening and tristeza because symptoms of both the diseases were prominent in almost all the locations. But for further confirmation of greening, disease molecular assay has to be performed (Figures 1 and 2).

Biological indexing

Bud wood from 30 trees, chosen at random and not necessarily showing symptoms, was used for indexing by the method of Roistacher (1991). Two indicator seedlings (*Citrus aurantifolia* that is, Maxican lime or Kagzi lime and *C. limon* to test as indicator hosts against CTV) were inoculated with buds obtained from CTV infected plants which were already tested as CTV positive using ELISA following the technique used by Wisler et al. (1996). Plants were grown in a greenhouse at about 27°C and inspected at least once each week for symptoms, beginning 15 days after inoculation. One bud was grafted onto each of the indicator plants. The plants were monitored for the symptom expression such as leaf yellowing and vein clearing. Fifteen (15) numbers of 1 year old seedlings of Assam lemon (*C. limon*) and 15 numbers of Kagzi lime or Maxican lime (*C. aurantifolia*) seedlings were grown inside the green house. Seedlings were tested by DAS-ELISA to test CTV negativity and were maintained in the greenhouse. The bud wood of 3 to 4 mm in diameter was budded at 20 cm height to each of the indicator seedlings. CTV infected scion of pencil thickness were collected from different fields nearby Jorhat, Assam. Trees from which bud sticks were taken were pre-tagged with CTV positive through DAS-ELISA. The samples were collected in the polypropylene bags. The scions were selected in such a way that every scion consisted of at least 2 to 3 swollen buds. Before putting the scion samples in polypropylene bags, these were wrapped in water soaked cotton so that they remain fresh during transportation. Then they were kept in refrigerator at 4°C until budding was performed and the process was completed within 48 h of their collection (Figure 3).

Double antibody sandwich-enzyme-linked immuno-sorbent assay (DAS-ELISA)

The leaf midrib tissue samples from different citrus growing areas were tested using DAS-ELISA as described by Clark and Adams (1977). The antibodies were obtained from Bioreba AG, CH4153, Reinach BL1, Switzerland. CTV IgG (20 µl) were diluted in 20 ml of coating buffer to coat each 96 well ELISA plate at the rate of 200 µl per well. The plate was incubated for 4 h at 30°C followed by three times washing with phosphate buffered saline Tween 20 (PBS-T). One gram (1 g) of leaf vein tissue from each sample was ground using mortar and pestle in 1 ml PBS-T. A 200 µl of the sample were added to each well. The plates were incubated over night at 4°C. ELISA plate was then washed three times with PBS-T. Following this, the enzyme conjugate was added to each well and the plate was then incubated for 4 h at 30°C. The plate was then washed three times with PBS-T. After washing, 5 mg p-nitrophenol

Table 1. Number of suspected CTV infected citrus leaf samples collected from different districts during 2009-2010.

S/N	Districts	Month of Survey (2009 - 2011)	Assam lemon (years)			Gul nemu (years)			Khasi mandarin (years)			Total
			<10	10 - 15	>15	<10	10 - 15	>15	<10	10 - 15	>15	
1	Golaghat	April, 2010 and September, 2010	13	10	2	9	1	11	8	4	20	78
2	Jorhat	December, 2009	8	10	-	3	8	1	5	3	8	46
3	Tinsukia	September, 2009	12	8	-	4	8	2	14	4	10	62
4	Lakhimpur	April, 2010	12	3	3	3	13	2	2	5	8	51
5	Karbi Anglong	April, 2010	7	2	2	3	5	1	3	2	16	41
6	N.C. Hills	April, 2010 and August, 2010	6	5	4	6	6	4	-	4	6	41
7	Kamrup	May, 2010	2	2	10	1	8	6	5	5	5	44
8	Kokrajhar	December, 2009 and July, 2010	2	3	10	5	5	8	4	4	7	48
Total			62	43	32	34	54	35	41	31	80	411

(DAS-ELISA).

Table 2. Symptoms expression in biological - indexing of *Citrus tristeza* virus (CTV) in indicator hosts.

Indicator host	No of symptomatic plant/no. of plant inoculated	No of asymptomatic plant/no. of plant inoculated	%Symptom expression	Symptoms produced (number of plants)		Symptoms appeared after weeks of inoculation
				VC	LY	
KL	6/10	4/10	60	4	4	5 - 9 week
AL	3/10	7/10	30	2	3	7 - 9 week

LY, Leaf yellowing; VC, vein clearing; KL, Kagzi lime; AL, Assam lemon.

phosphate substrate tablets were dissolved 20 ml of diethanolamine buffer and 200 µl of substrate was added in each well and observed for change of colour in the wells. CTV IgG, enzyme conjugate and substrates were purchased from Bioreba (USA). ELISA plate was then read by the ELISA plate reader (Bio Rad) using 405 nm wavelength after 1 h of addition of substrate. Plants were considered infected with CTV if the ELISA reading was four times higher the average reading of the healthy samples (usually ≥0.1) (Azzam et al., 2001). Data were analyzed and disease incidence was recorded.

RESULTS AND DISCUSSION

Biological indexing

The results of biological indexing assay on two indicator hosts viz., Kagzi lime or Maxican lime *(C. aurantifolia)* and Assam lemon (*C. limon*) are shown on Tables 2 and 3. Both leaf yellowing and vein clearing symptoms were observed on both the hosts from 5th to 9th weeks after inoculation (Table 2). The results revealed that among the two species of citrus used as indicator hosts, Kagzi lime (*C. aurantifolia*) showed better expression of symptoms (60%) compared to Assam lemon (30%). The inoculated plants after 9th week (63 days) again tested using DAS-ELISA assay. The results revealed that percentages of positive plants were 90% in Kagzi lime

followed by Assam lemon (80%). The comparison analysis between biological indexing and immunological indexing further indicated that although biological indexing was accepted as one of the reliable method of virus identification, the DAS-ELISA assay had been found more accurate and quick in confirming virus incidence (Table 3).

Identification of plant tissues as source of virus

DAS-ELISA of leaf samples from eight districts of Assam on three different *Citrus* species was performed successfully and ELISA value (Virus titre values) ranges were recorded (Table 4). The results of DAS-ELISA showed 261 trees, out of 411 tested, infected with CTV indicating 63.50% CTV disease incidence in Assam. Results revealed presence of CTV in all the surveyed districts of Assam showing a high incidence of 78.04% CTV in Karbi Anglong district followed by 76.92% in Golaghat district (Table 5). Among the three different *Citrus* species, Assam lemon showed maximum incidence of CTV with 76.47% followed by Khasi mandarin (61.18%) and Gul nemu (52.03%). The results also revealed that in all the three different *Citrus* species Assam lemon, Gul nemu and Khasi mandarin the percentage of CTV infection was more in the higher age

Table 3. Comparison of biological - indexing and double antibody sandwich-enzyme-linked immuno-sorbent assay (DAS-ELISA) against CTV on indicator hosts.

Citrus species	Percentage of infection after 9 weeks (63 days)	
	Biological - indexing	DAS-ELISA
Assam lemon (AL)	30	80
Kagzi lime (KL)	60	90

Table 4. Accumulation of *Citrus tristeza* virus (CTV) titer in CTV infected *Citrus* leaf samples in different citrus growing districts of Assam.

District	Range of OD_{405} values	OD_{405} values in healthy control
Jorhat	0.66 - 1.16 (3 - 5)	0.24
Tinsukia	1.12 - 2.59 (3 - 7)	0.38
Golaghat	1.07 - 1.46 (2)	0.63
Lakhimpur	0.84 - 2.39 (3 - 9)	0.28
Karbi Anglong	0.45 - 0.86 (2 - 4)	0.24
N.C. Hills	0.59 - 1.88 (2 - 8)	0.25
Kamrup	0.28 - 1.14 (1 - 5)	0.25
Kokrajhar	0.55 - 1.29 (3 - 6)	0.21

OD value was measured at 405 nm. Data in parentheses represent CTV titer in folds compared to healthy control.

Table 5. Over all *Citrus tristeza* virus (CTV) infection in leaf midrib tissues of different citrus species in Assam using DAS-ELISA.

S/N	Districts	Locations	% Infection			
			Assam lemon	Khasi mandarin	Gul nemu	Average
1	Golaghat	Bokeal Buragohain Khat, No. 2 Chatiana (Bogijan), Naojan, Bhilgaon, Namlagua gaon, Missamora	23/25 (92.0)	24/32 (75)	13/21 (61.90)	60/78 (76.92)
2	Jorhat	Nagajanka, AAU Orchard, Choladhara	12/18 (66.6)	9/16 (56.26)	6/12 (50.00)	27/46 (58.69)
3	Tinsukia	Kachijan, Borgaon, Kherjan, Hatigarh, Kakapathar	12/20 (60.0)	13/28 (46.42)	8/14 (57.14)	33/62 (53.22)
4	Lakhimpur	Hatilong, Town Bantow, Nowboisha, Khelmati, Chutiakari, Johing	16/18 (88.8)	12/15 (80)	11/18 (61.11)	39/51 (76.47)
5	Karbi Anglong	Singnot Engti Gaon, Merabheti Kaliani, Thengal Gaon (Nilip Block), Diphu	6/11 (54.54)	20/21 (95.23)	6/9 (66.66)	32/41 (78.04)
6	N.C. Hills	Haflong, Sub centre Area, Guruling area	10/15 (60.0)	5/10 (50)	7/16 (43.76)	22-41 (53.65)
7	Kamrup	HRS (Kahikuchi), Mirza, Sonapur	13/14 (92.8)	0/15 (0.00)	4/15 (26.66)	17/44 (38.64)
8	Kokrajhar	Tilapara, Debargaon, Gossaigaon, Kachikotha Hainary, Chirang	12/15 (80.0)	10/15 (66.66)	9/18 (50.00)	31/38 (64.58)
	Total Infection (%)		104/136 (76.47)	93/152 (61.1)	64/123 (52.03)	261/411 (63.5)

groups viz., > 15 years and 10 to 15 years followed by lower age group (<10 years old plants) (Table 6).

CTV infection was detected in all the surveyed locations. This is an indication that CTV is prevalent in this region. Typical symptoms of CTV infection was observed in most of the orchards and gardens. Prevalence of the vector *Toxoptera* spp. was noticed in all the surveyed locations. The results of DAS-ELISA

Table 6. Status of infection of *Citrus tristeza* virus (CTV) in different citrus hosts with age of the plant.

Citrus species	Age group (years)	Percent CTV infection	Average percent infection
Assam lemon	Up to 10	41/61 (67.2)	
	10 to15	35/41 (85.3)	104/136 (76.47)
	Above 15	28/32 (87.5)	
Gul nemu	Up to 10	10/31 (32.2)	
	10 to 15	30/51 (58.8)	64/123 (52.03)
	Above 15	24/32 (75.0)	
Khasi mandarin	Up to 10	11/38 (28.9)	
	10 to 15	18/29 (62.0)	93/152 (61.18)
	Above 15	64/80 (80.0)	
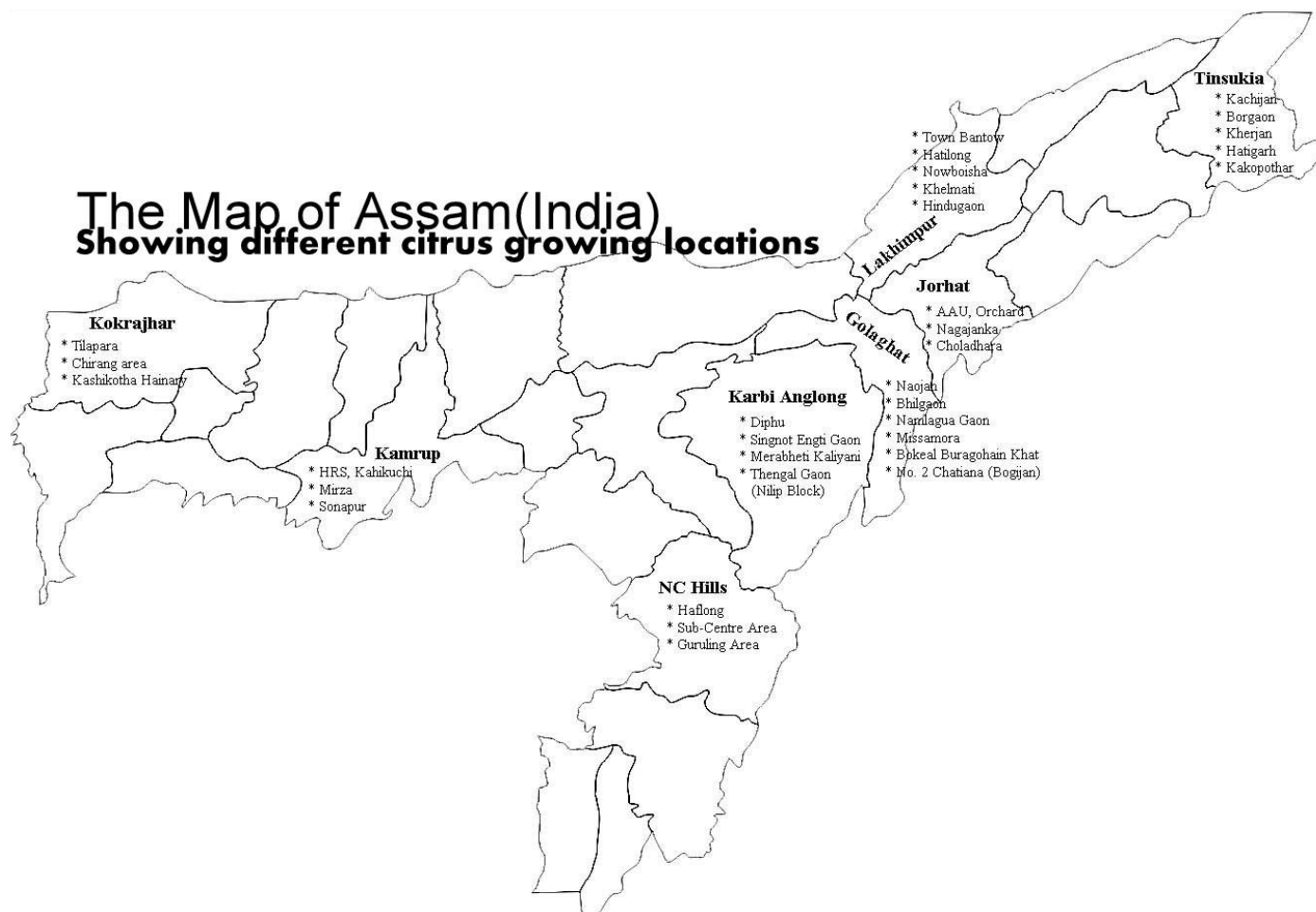
Figure 1. Map of Assam (India) showing different citrus growing locations.

showed 63.50% CTV disease incidence in Assam at different level of incidence in different survey sites. DAS-ELISA results also revealed that in all the three different *Citrus* species Assam lemon, Gul nemu and Khasi mandarin, the percentage of positivity is more in the higher age group. This indicates that incidence of CTV is more in matured and older plants. The results further confirm the earlier report of incidence of CTV in the citrus
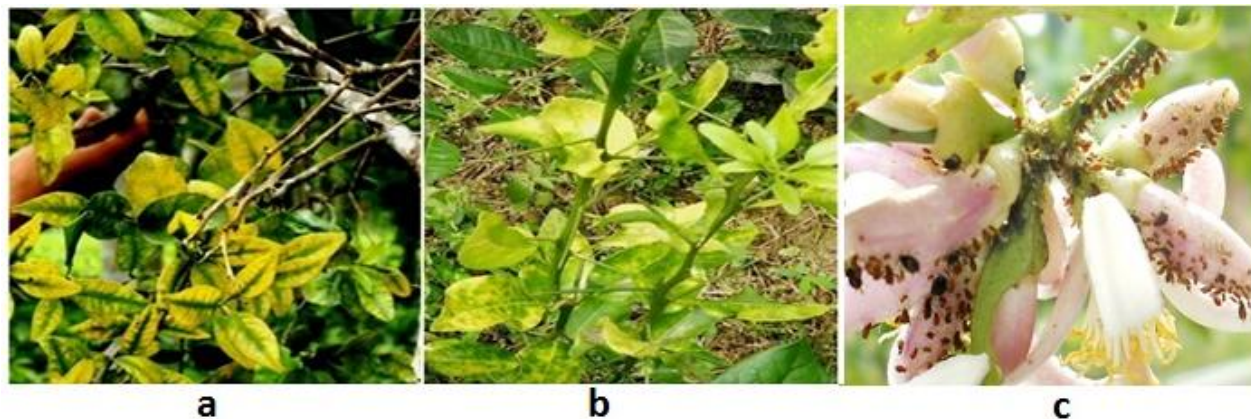
Figure 2. Symptoms of CTV in Khasi mandarin: **a,** >10 years old plant; b, <10 years old plant; c, insect vector *Toxoptera citricida* in the field.

Figure 3. Process of biological indexing: a, Indicator host *Citrus aurantifolia*; b, Performing budding process; c, Indicator plants after budding.

growing areas of Assam (Bhagabati et al., 1989). Similar type of studies were carried out by Kishore et al. (2010) who studied the assessment of CTV incidence in mandarin of Sikkim, estimated by DAS-ELISA. Biswas (2008) also diagnosed CTV in Darjeeling Hills through molecular tools and reported CTV infection up to 90.00% in mandarin trees at varying altitudes.

Conclusion

CTV is the most important and widely distributed disease in Assam. Laboratory based ELISA test could be successfully used to detect CTV infection in the field. Thus, findings of the research would be helpful in initiating future strategies on CTV certification, eradication, rejuvenation and management programme.

Conflict of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

REFERENCES

Azzam O, Imbe T, Ikeda R, Nath PD, Coloquio E (2001). Inheritance of

resistance to rice tungro spherical virus in a near isogenic line derived from Utri Merah and in rice cultivar TKM6. Euphytica 122(1):91-97. http://dx.doi.org/10.1023/A:1012605218343

Bar-Joseph M, Garnsey SN, Gorsalves D (1979). The closteroviruses: A distinct group of elongated plant viruses. Adv. Virus Res. 25:99-168. http://dx.doi.org/10.1016/S0065-3527(08)60569-2

Bar-Joseph M, Marcus R, Lee RF (1989). The continuous challenge of *Citrus tristeza* virus control. Annu. Rev. Phytopathol. 27:291-316. http://dx.doi.org/10.1146/annurev.py.27.090189.001451

Bhagabati KN, Ahlawat YS, Chakraborty NK, Borthakur BC (1989). Distribution of greening, tristeza and mosaic disease of citrus in North Eastern States of India. Indian Phytopathol. 42:552-555.

Biswas KK (2008). Molecular diagnosis of cirus tristeza virus in mandarin (*Cirus reticulata*) orchards of Darjeeling hills of West Bengal. Indian J. Virol. 19:26-31. http://dx.doi.org/10.1007/s00705-010-0602-7

Biswas KK (2010). Molecular characterization of cirus tristeza virus isolates from the Northeastern Himalayan region of India. Arch. Virol. 155:959-63.

Cambra M, Moreno P, Navarro L (1979). Rapid detection of *Citrus tristeza* virus by ELISA. Anales INIA, *Serie.* Prot. Vegetal. 12:115-25.

Capoor SP, Rao DG (1967). Tristeza irus infection of citrus in India. In : Proc. Int. Symp. or Subtrop. Trop. Hort. Bangalore pp. 723-736.

Chakraborty NK, Ahlawat YS (2001). Indexing of cross protected and non-cross protected citrus cultivars/species for citrus tristeza virus by enzyme linked immunosorbent assays. Plant Dis. Res. 16:10-6.

Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. J. General Virol. 34:475-483. http://dx.doi.org/10.1099/0022-1317-34-3-475.

Edson B, Aranzazu M, Nieves C, Antonio O, de Ana L, Eduardo V, Jordi PP, Mariano C (2008). Quantitative detection of *Citrus tristeza* virus in plant tissues and single aphids by real-time RT-PCR. Eur. J. Plant Pathol. 120:177-88. http://dx.doi.org/10.1007/s10658-007-9206-9

Ghosh SP (2007). Citrus fruits. ICAR, New Delhi.

Karasev AV, Boyko VP, Gowda S, Nikolaeva OV, Hilf ME, Koonin EV, Niblett CL, Cline K, Gumpf DJ, Lee RF (1995). Complete sequence of the *Citrus tristeza* virus RNA genome. Virology 208:511-20. http://dx.doi.org/10.1006/viro.1995.1182

Kishore K, Rahman H, Kalita H, Pandey B, Monika N (2010). Prevalence of *Citrus tristeza* virus in mandarin of Sikkim Himalayan Region. Indian J. Virol. 21:140-143. http://dx.doi.org/10.1007/s13337-010-0019-7

Verma NN, Ghosh SP (1979). Citrus is North-East India. Indian Hort. 23:2-4.

Full Length Research Paper

Economic empowerment of Scheduled Caste (SC) landless rural women through mushroom cultivation: A case study

Singh J[1]*, V. P. Chahal[2], Rathee A[3] and Singh K[4]

[1]Extension Education Unit, CCSHAU, Krishi Vigyan Kendra (KVK), Sonipat, India.
[2]Division Agricultural Extension, ICAR, KAB-I, New Delhi, India.
[3]Farm Management Unit, CCSHAU, KVK, Sonipat, India.
[4]Department of Agricultural Engineering, CCSHAU, KVK, Sonipat, India.

Krishi Vigyan Kendra (KVK) Kurukshetra took up an initiative for economic empowerment of landless Scheduled Caste (SC) rural women by motivating them to adopt mushroom cultivation as an income generating activity. As part of this programme, KVK has conducted three trainings for these landless SC rural women since 2008-2009. In total, 120 such ladies were trained over a period of 3 years. The trainees were provided practical training through method demonstrations on compost preparation, spawning and casing management practices, harvesting and packaging of button mushroom. The post-training evaluations of these 120 women from 12 villages showed that majority (>75%) of them gained low to medium level of knowledge on the vocation. These trained women had taken up white button mushroom cultivation in polythene bags during October to March every year. The economic empowerment of the landless SC rural women by way of mushroom production of KVK has created awareness regarding its cultivation among them. With technical back-up of the KVK, the adoption of mushroom cultivation by 25% of trained landless SC rural women has paved the way for their economic empowerment. A sense of belongingness, thus, has been created among these women towards mushroom farming. As many as 50% of them have found it a good livelihood option for generating additional income for the family. In the coming years, mushroom farming by landless SC women in the district will become an integral part of socio-economic development process and a low cost self-help based exemplary model of economic empowerment. In order to sustain this model, the women opined that further convergence of different actors is essential for provisions of adequate micro-credit, assured market, family support, easy availability of pasteurized compost and casing material and facilities for value addition and processing of mushroom. There is, only one pasteurized compost and casing material making unit operational in Kurukshetra district.

Key words: Mushroom, economic empowerment, Scheduled Caste, rural women.

INTRODUCTION

Mushroom is highly nutritious food containing protein, iron, vitamins and salts ideally required for heart and

diabetic patients. Further, with the increasing number of people refraining from non-vegetarian foods, mushroom serves as the best alternative because of its high nutritional and calorific constituents. Its farming is remunerative with quick return in a short span of time. It is grown on farm waste, require less land, provide income and work in lean period of October to March when less agricultural operations are performed (Hari et al., 2008).

In India, 280 edible species are produced with production of 85000 metric tons (Kokate et al., 2010). Haryana State ranks third in mushroom production. The major mushroom producer districts in Haryana are Sonipat, Panipat and Kurukshetra.

More than 90% of rural women in India are unskilled. The work participation rate among Scheduled Castes (SC) in India is about 25% only. Among main SC workers about 50% are agricultural laborers and majority of them (>65%) are rural women (National Commission for Women, 1998). The landless SC rural women in Haryana State are also deprived of regular work opportunities. The situation of such women in Kurukshetra district of the State is not different. The major income activity of such women hinges around the agricultural work.

There is considerable potential for cultivation of white button mushroom in Kurukshetra district of Haryana, if scientific technology is adopted and provided to the farmers. Keeping in view, Krishi Vigyan Kendra (KVK) Kurukshetra has organized more than 50 skills based training programmes in white button mushroom cultivation covering more than 1000 farmers and farm women. Subsequently, KVK in Kurukshetra district of Haryana took up an initiative for economic empowerment of landless SC rural women by motivating them to adopt mushroom cultivation as an income generating activity and thus, trained 40 women of different villages belonging to self-help groups (SHGs) in the year 2008-2009.

MATERIALS AND METHODS

As part of SC development programme, the KVK, Kurukshetra has started providing vocational training on cultivation of button mushroom to 40 SC women each year since 2008-2009. Since then 120 SC women of different villages belonging to SHGs have been trained. The women were given practical training through method demonstrations on compost preparation, spawning, casing, management practices, harvesting and packaging of button mushroom. The post-training evaluations of 120 women from villages viz; Antehri, Yaari, Dhurala, Bodla, Dayal Nagar, Bhore Saidan, Sunaria, Ahmedpur, Sirsama, Sanghour and Muradnagar were conducted and their responses recorded on different aspects. No prior training was obtained by the respondents in mushroom cultivation. The post training analysis showed that there is increase in production and productivity of mushroom. Further, marketing skills gained in the training helped them in getting higher prices for mushroom with wider market reach and improved post-harvesting skills and reduce post-harvest looses. Group marketing with increased bargaining power with producers also adds on to their skills. The training need of the mushroom growing women has been operationalized as an expression of need for training in selected

areas as felt by them in the study. It was measured in terms of the expressed opinion of the respondents on three point continuum that is, most needed, somewhat needed and least needed, the scores assigned were 3, 2, and 1, respectively. The data so recorded was tabulated and analyzed with proper statistical tools.

RESULTS AND DISCUSSION

Respondents' profile

The data presented in Table 1 shows that 37.5% of the respondents were below 30 years of age followed by middle and old aged. Further probing the data, it was found that 32.5% of them are illiterate followed by can write name only. It was also found that they live in nuclear family and have up to four members in their family. Therefore, it can be concluded that majority of women are in young age group, illiterate, landless labours, living in nuclear family and belong to low income group.

Motive to grow mushroom

The data presented in Table 2 shows that the main motive of mushroom growers is to take cheaper loan (69.1%) and advantage of government schemes (68.3%), while, some of them also expressed economic independence (31.2%) and recognition in society and family (23.3%) as their motive to grow mushroom in groups.

Mushroom units established

The data presented in Table 3 shows that 25% of trained women adopted mushroom cultivation in successive years and earned a handsome amount of profit from it.

Participants' gain in knowledge

It was found that majority of the participants gained low to medium level of knowledge on the vocation and opined for further training on some of the aspects of mushroom cultivation (Table 4). The low to medium level in knowledge gain shows that the participants are not fully trained, and there is scope for skill upgradation. The scores gained in different cultivation aspects were categorized in low, medium and high level of knowledge gain.

Economic analysis

As a part of the programme, each trainee-woman was given 85 to 105 bags of spawned pasteurized compost and casing material prepared by District Mushroom Growers Cooperative Society. Most of the women started

Table 1. Profile of the respondents.

S/No.	Attribute	Number	Percentage
	Age		
1	Up to 30 years	45	37.5
	31 - 50 years	57	47.5
	>50 years	18	15.0
	Education		
2	Illiterate	39	32.5
	Can write name only	32	26.7
	Primary	26	21.7
	Matric	19	15.8
	Graduate	04	03.3
	Family type		
3	Joint	37	30.83
	Nuclear	83	69.17
	Family size		
4	Up to 4 members	62	51.67
	>4 members	58	48.33
	Family income/season		
5	>Rs. 20,000/-	43	35.83
	Rs. 20,000 to 50,000	61	50.83
	< Rs. 50,000	16	13.34
	Social participation		
7	Members of SHGs	120	100.00
	Member of panchayat	04	3.33

N = 120.

Table 2. Motive to grow mushroom in groups.

S/No.	Reasons	Frequency	Percentage
1	To become economically independent	38	31.2
2	For recognition in family and society	28	23.3
3	For better future	102	85.0
4	To take advantage of government initiatives	82	68.3
5	To get loans	83	69.1

*Multiple response found. N=120.

mushroom production unit either in the house backyard by raising a thatched hut of bamboo and paddy straw or in their home itself as they do not own even a small piece of land. In order to provide continuous technical backup to the first time women mushroom growers, the KVK expert regularly visited units to guide and solve their problems on the site. With this intervention the landless SC rural women were able to produce up to 18 kg of button mushroom/100 kg of pasteurized compost and earned Rs 6719.5 and Rs 525.0 per member highest and lowest, respectively during the season. The average cost of cultivation was found to be Rs 25.0/kg, while the sales price of mushroom in the local market varied from Rs 40 to 60/kg. However, it is reported that sales price of mushroom went up to Rs 100/kg, when production is low and demand is high due to marriages and other social

Table 3. Year-wise adoption of mushroom cultivation in subsequent years.

S/No.	Year	Participants	Participants continue mushroom growing in next year	Continue percentage
1	2008 - 2009	40	10	25.00
2	2009 - 2010	40	11	27.50
3	2010 - 2011	40	09	22.50

Table 4. Gain in knowledge.

S/No.	Categories	Number	Participants
1	Low	21	17.50
2	Medium	69	57.50
3	High	30	25.00
	Total	120	100.00

N = 120.

Table 5. Mushroom production and economic returns[*] of SHGs.

S/No.	Name of SHG	Village	Pasteurized compost (kg)	Mushroom production (kg)	Expenditure	Income (Rs)	Net profit (Rs)	Net profit/ Member (Rs)
1.	Ravi Dass (9)	Yaari	8550	1424	47955	71250	23295	2588.0
2.	Ekta (12)	Antehri	11400	2166	71250	10830	37050	3087.5
3.	Sant Ravi Dass (10)	Antehri	9000	2003	130195	63000	67195	6719.5
4.	Jai Maa (11)	Dhurala	10450	1985	61610	97265	35655	3241.0
5.	Hariom (10)	Dhurala	2850	527	17500	26350	9850	985.0
6.	Bhole Nath (10)	Dhurala	2850	475	17300	23275	5975	597.5
7.	Jai Santosi Maa (13)	Bodla	12285	2389	155269	85995	69274	5328.7
8.	Sri Ganesh (7)	Dayal Nagar	6615	735	36750	40425	3675	525.0
9.	Bhairav Baba(7)	Bhore Saidan	6615	1102	46305	71663	25358	3622.5
10.	Sunaria (7)	Sunaria	6615	1029	46305	66885	20580	2940.0
11.	Bala Sundri (6)	Ahmedpur	5670	9921	39690	694610	29750	4958.3
12.	Ambedkar (7)	Sirsama	6615	1176	46305	83496	37191	5313.0
13.	Kastoorba (7)	Sanghour	6615	956	46305	69788	23483	3354.7
14	Kali Maa (4)	Muradnagar	3780	588	26460	40572	14112	3528.0

*Total members= 120; Gross profit = Rs.402443; Average profit per member = 3353.7. N =120.

functions in the area. The average price per kg of mushroom received is Rs. 50. Goyal (2006) showed similarity with results presented (Table 5).

Average net profit of Rs. 3353.7 per member handsomely provides leverage to the SC women. Thus, extra income resulted into increased expenditure on solely as per the wishes of women growers.

This has resulted into improved living standard, better health care, increased expenditure on household durables and cloths.

Perceived attributes of success

The data presented in Table 6 clearly indicated that family is the basic force behind their success and supportive behaviour of husband has also played an important role.

Training needs

The data pertaining to the training needs of the mushroom growers presented are in Table 7.

It shows that they require further training on compost preparation followed by casing mixture preparation as these two operations are highly technical and the success of the unit heavily depends on it.

Table 6. Perceived factors of success.

S/No.	Factor	Rank
1	Husband	II
2	Family	I
3	Relatives	IX
4	Self confidence	III
5	Friends	X
6	Government officials including KVK	IV
7	Finance available	VIII
8	Easy availability of pasteurized compost	V
9	Marketing facility	VI
10	Government initiatives	VII

Table 7. Training Needs.

S/No.	Technological aspect	Mean score	Rank
1	Compost preparation	2.58	I
2	Casing mixture preparation	2.42	II
3	Management of environmental parameters (Temp./rh/Co)	2.33	III
4	Management of pest/diseases/disorders	2.29	IV
5	Harvesting operation	1.63	VI
6	PHT (packaging etc.)	1.42	VII
7	Marketing intelligence/ information	1.71	V

N = 120.

Conflict of Interest

The author(s) have not declared any conflict of interest.

REFERENCES

Goyal SP (2006). Dissemination of mushroom production technology. In: The challenges in agriculture development- role of KVKs. Directorate of Extension Education, CCSHAU, Hisar, India. P. 73.

Hari Om, Chauhan RS, Malik RK, Singh VP, Singh Dilbag, Lathwal OP, Goyal SP, Yadav SK, Sher Singh, Malik HR (2008). Diversification through Farming System Approach. Technical Bulletin (30), Krishi Vigyan Kendra, Kurukshetra and Directorate of Extension Education, CCSHAU, Hisar, India P. 56.

Kokate KD, Rathi Anil, Narula AM, Keshava (2010). Mushroom Farming. Zonal Project Directorate, Zone- 1, PAU Campus, Ludhiana-141004.

Report on National Commission for Women (1998). National Commission for Women, New Delhi, India.

Full Length Research Paper

Chitosan/kudzu starch/ascorbic acid films: Rheological, wetting, release, and antibacterial properties

Xiaoyong Song[1]* and Luming Cheng[2]

[1]North China University of Water Resources and Electric Power 36 Beihuan Road Zhengzhou, P. R. 450011 China.
[2]The Second Hospital Affiliated to Zhengzhou University, Zhengzhou 450014, China.

Chitosan was blended with kudzu starch to prepare edible composite films adding ascorbic acid as antioxidant and Tween 20 as surfactant, and choosing acetic acid, lactic acid and malic acid as solvents. Rheology and wettability of film forming solutions, and antibacterial, release and physical properties of the composite films were investigated. Results showed that, acid solvent types significantly affected the rheology of film forming solutions but hardly had influence on wettability. The growth of *Escherichia coli and Staphylococcus aureus* were inhibited by the composite films, and the release process of ascorbic acid were effectively delayed. Among the composite films, film prepared by acetic acid had the strongest mechanical strength and the best controlled-release effect. Film choosing lactic acid as solvent had the best flexibility. The water vapor permeability coefficients of film using malic acid to dissolve was the smallest, and its antibacterial ability was the highest.

Key words: Chitosan/kudzu starch/ascorbic acid edible film, rheological properties, wettability, antibacterial properties, release behaviors, physical properties.

INTRODUCTION

In recent years, more attention has been paid to biopolymer-based edible food packaging as a potential alternative to synthetic polymer-based packaging materials which cause negative environmental impact due to their non-biodegradability.

Biopolymers, such as carbohydrates, proteins and lipids can fulfill the requirements for preparing edible films and these materials can be utilized individually or as mixed composite blends. These edible polymer films can totally cover food surfaces, form barriers against various small molecules including oxygen, moisture, aroma and oil, reduce mechanical injuries, and thus prevent food quality deterioration and preserve/improve food integrity

(Siracusa et al., 2008). Besides, edible films can be chosen as carriers of many functional additives (such as antibacterials, antioxidants, flavors, and colorants) which enhance the functionality of the packaging materials (Martins et al., 2012). Nice edible films should be designed to meet a number of requirements, such as having proper mechanical properties, good appearances (adequate gloss and transparency) and favorable gas barrier properties (Pitak and Rakshit, 2011).

Among the biopolymers for formulating edible films, starch is one of the most promising raw materials owning to its biodegradability, abundance, low cost, renewability and film-forming ability (Averous et al., 2001;

*Corresponding author. E-mail: songxiaoyong@ncwu.edu.cn. Tel:/Fax: +86 371 65790043.

Peressini et al., 2003). Furthermore, starch based films have been particularly considered because their physical properties are similar to synthetic polymers: transparent, tasteless, odorless, semi-permeable to CO_2 and resistant to O_2 passage (Nisperos-Carriedo, 1994; Skurtys and Dieulot, 2013; Woggum et al., 2014). Many researchers have studied the potential applications of starch edible films and confirmed their availability to extend the shelf life of fresh and minimal processed products (Bergo et al., 2008; Bertuzzi et al., 2007; Talja et al., 2007).

However, starch based films show several disadvantages, e.g. strong hydrophilic properties and inadequate mechanical characteristics (Chillo et al., 2008). In order to overcome the above shortcomings and improve the functional properties of starch films, blending with other biopolymers is a promising strategy (Flores et al., 2007; Gerschenson and Goyanes, 2009; Vásconez et al., 2009).

Chitosan is a natural polymer derived from deacetylation of chitin (Abugoch et al., 2011). Chitosan possesses immense potential as a packaging material in food industry owing to its nontoxicity, biodegradability, biocompatibility, antimicrobial activity and film forming capacity (Abugoch et al., 2011). In addition, chitosan films have low oxygen permeability coefficients, good mechanical properties which insure their widely applications (Avila-Sosa et al., 2012; Bonilla et al., 2012). Some reports have indicated that starch and chitosan can form nice composite films which possess better properties than films using them alone (Bourtoom and Chinnan, 2008; Vásconez et al., 2009). However, chitosan is only soluble in some acid solutions and acid types can significantly affect the behaviors of chitosan based films (Kim et al., 2006).

In addition, incorporating natural preservatives (such as antioxidant and antimicrobial materials) into edible films is an effective alternative to reduce the dosage of chemical preservation agent. These active additives that enhanced films can keep ensuring the safety of food surfaces through the controlled release of active substances (Devlieghere et al., 2004; Fatih et al., 2009), which display obvious advantages over the direct application of preserving agents, e.g. smaller agent dosage is needed to achieve a target shelf life. Ascorbic acid is one of the most extensively used additives as a dietary supplement of vitamin C and an antioxidant to protect the food quality (Bastos et al., 2009).

To the best of our knowledge, there is little comprehensive study of the effects of different acid-solvents on the rheology, and wettability of chitosan based film-forming-solutions (FFSs), in addition to the release behaviors of active additives from films. In this present paper, chitosan was blending with kudzu starch, a traditional healthy food in China to cast composite films choosing ascorbic acid as additive. The effects of three acid solvents (acetic acid, lactic acid, and malic acid) on the wettability and rheological properties of FFSs were studies, besides, the antibacterial, physical and controlled-release properties of edible films were also considered in order to provide useful knowledge of its possible applications in prolonging food shelf life.

MATERIALS AND METHODS

Kudzu starch (*Pueraria lobata*, water content: ca.14%, amylose: ca.30%) was purchased from Xichuan Chunyu Geye Biotechnology Co., Ltd. (Henan, China). Chitosan (molecular weight: ca. 420 kDa, deacetylated degree: 88.5%) was purchased from AK Biotech Ltd. (Shandong, China). Glycerol, ascorbic acid, acetic acid, lactic acid, DL–malic acid, anhydrous calcium chloride and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tween 20 ($C_{58}H_{114}O_{26}$) obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) was used as surfactant to increase the wettability of the FFSs. Luria–Bertani broth (LB) and tryptone soy broth (TSB) were purchased from Qingdao Hope Bio-technology Co., Ltd. (Shandong, China). *Escherichia coli* CMCC44102 and *Staphylococcus aureus* ATCC6538 were kindly provided by the lab of department of Food Science and Technology, Shanghai Jiao Tong University (Shanghai, China).

Film preparation

Kudzu starch was dispersed in deionized water to obtain 2% (w/v) kudzu suspension. The solution was heated at about 100°C for 15 min under stirring to accomplish a complete starch gelatinization, during the process glycerol (0.6%, w/v) and tween 20 (0.1%, w/v) were added. 2% (w/v) of chitosan solution was prepared by dispersing chitosan in 1% (w/w) of acetic acid, lactic acid or malic acid solution and stirring with a magnetic stirrer (Shanghai Huxi analysis instrument factory Co., Ltd., Shanghai, China). In order to achieve more flexible films, glycerol (0.6%, w/v) and tween 20 (0.1%, w/v) were added. After chitosan was dissolved completely, the solution was filtered with cheesecloth.

Chitosan/kudzu suspensions were prepared by mixing 100 ml of chitosan solution and 100 ml of suspension together. All the solutions were cooled to room temperature and adjusted to pH = 4.0 with corresponding acid (Vásconez et al., 2009). Then, 0.5% (w/v) of ascorbic acid was added and the solutions were stirred for 5 min, and pH was measured, adjusted and measured again. After homogeneous processing, the FFSs were transferred into a vacuum oven for about 1 h at room temperature to remove the air bubbles.

About 200 ml of solutions were casted over the leveled glass plates (25 × 25 cm) and dried at room temperature for at least 16 h until the weights approached to constant values giving films with ca. 0.1 mm thickness. Thereafter, the films were carefully peeled from the plates and stored for 48 h in a constant temperature and humidity chamber (KBF720, Binder, Germany) at 50% relative humidity and 25°C before further tests. Chitosan/kudzu starch/ascorbic acid composite film (solution) was recorded as Ch-Ku-As. The composite film (solution) as chitosan dissolved in acetic acid, lactic acid, and malic acid were recorded as Ch-Ku(Aa)-As, Ch-Ku(La)-As and Ch-Ku(Ma)-As, respectively.

Contact angle

The measurement of the contact angles of FFSs on the clean leveled surface of sized glass and paraffin wax substrates were performed using a Contact Angle Analyzer (DSA 30, Kruss Co., Germany) at room temperature. Six replicate determinations were carried out for each sample, and the results were averaged.

Rheological measurements

Rheological tests of degassed FFSs were performed by a controlled-stress rheometer (Brookfield R/S–CC, Brookfield Engineering Laboratories, Inc., Massachusetts, USA) using CC25 coaxial cylinder sensor. The measurements were conducted at 25 ± 0.5°C controlled by circulating water. For steady-shear measurements, all the samples were sheared continuously at shear rates ranging from 0 to 1000 s^{-1}, using 10 min to reach the maximum shear rate and another 10 min back to zero shear rate. The apparent viscosity was recorded as a function of shear rate. The Ostwald de Waele model (Equation 1) was applied to determine the consistency index and flow-behavior index, a dimensionless number that indicates the closeness to Newtonian flow. It takes a value of 1 for Newtonian, between 0 and 1 for pseudoplastic fluids and higher than 1 for dilatant fluids (Xiao et al., 2012).

$$\sigma = k \cdot \gamma^n \tag{1}$$

Where σ is the shear stress (Pa), γ is the shear rate (s^{-1}), k is the consistency index (Pa s^n), and n is the dimensionless flow behavior index.

Antibacterial tests

Antibacterial tests of Ch-Ku-As films were conducted by liquid culture method (Bajpai et al., 2010), with *E. coli* and *S. aureus* as model bacteria. All the solutions and vessels used were sterilized before tests. The tests were conducted under sterile conditions and were run in triplicates. A loop of *E. coli* was inoculated into 25 ml LB whereas a loop of *S. aureus* was inoculated into 25 ml TSB, respectively. The bacteria suspensions were then incubated in a constant temperature vibrator (Taicang Experimental Equipment Plant, Jiangsu, China) with a shaking speed of 200 rpm at 37°C overnight. In order to display the inhibitory differences of Ch-Ku-As films more clearly, appropriate amounts of the overnight suspensions were again transferred into sterilized nutrient broths and incubated at 37°C to the exponential growth phase for subsequent tests (Fernandez-Saiz et al., 2009). The concentrations of the *E. coli* and *S. aureus* suspensions were 5×10^8 CFU/ml and 7×10^8 CFU/ml, respectively. For the liquid culture test, 50 ml of bacterial cell suspensions and 1.25 g (dry weight) of the Ch-Ku-As films were added into 100 ml flasks. The control assay was conducted without adding films. All the flasks were then incubated in constant temperature vibrator with a shaking speed of 200 rpm at 37°C. Suspension-aliquots were taken out every 15 min during experiment and measured the optical density (OD) at 600 nm to reflect the bacteria growth using a UV–2100 spectrophotometer (UNICO (Shanghai) Instruments Co., Ltd., Shanghai, China) until 90 min.

Release test

Release test was carried out based on the method described by Flores et al. (2007) with some modifications. Film discs (Φ = 1.4 cm) were put into weighing bottles (Φ 70 × 35 mm) containing 60 ml of distilled water and stirred magnetically at 150 rpm. The contents of ascorbic acid released to water during experiment was determined by the antioxidant activities in water which were monitored by taking 1 mL of samples at different time periods (1, 3, 5, 7, 9, 15, 20, 25, 30, 45, and 60 min) at 25°C and using DPPH decolorization assay to calculate. The release test was done in triplicate for each film.

We used Fick's second law to describe the release behavior of ascorbic acid (Mastromatteo et al., 2009).

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp[-\frac{(2n+1)^2}{L^2} \pi^2 Dt] \tag{2}$$

Where M_t was the ascorbic acid concentration at time t (g ascorbic acid/g film), M_∞ was the equilibrium ascorbic acid concentration (g ascorbic acid/g film), L was the thickness of film (m), D was diffusion coefficient (m^2/s), t was releasing time (min), n was the positive integer.

Water vapor permeability

The water vapor permeability (WVP) of film was determined gravimetrically based on the ASTM E96-95 (ASTM, 1995a) method with some modifications. The film thickness was measured at five randomly chosen points using a digital micrometer (with an accuracy of 0.02 mm) before test and the mean value was used to calculate WVP. The films were sealed on the top of permeation cells (diameter 21 mm and height 25 mm) containing granular (Φ < 2 mm) anhydrous calcium chloride. The cells were placed in desiccators containing saturated NaCl solutions at 25°C and provided 0/75% relative humidity gradient. The cells were weighed as a function of time until the steady state was reached using an analytical balance (±0.0001 g). WVP was calculated as follow:

$$WVP = \frac{m L}{A t \Delta P} \tag{3}$$

Where m is the weight of water permeated through the films (g), L is the average thickness of edible films (m), A is the permeation area (m^2), t is the time of permeation (s), and ΔP is the partial water vapor pressure difference across the two sides of the film (Pa). Five replicates were obtained for each test.

Mechanical properties

Mechanical properties of films were determined according to the method described by Chen et al. (2010) with some modifications and were carried out using a texture analyzer (TA-XTplus, Stable Micro systems, Surrey, UK) at room temperature. Eight repetitions were performed for each sample. During the test, rectangular films of 20 × 80 mm were mounted between the tensile grips (A/TG model) and stretched at a rate of 0.8 mm s^{-1} until breaking. Initial grip separation was set at 50 mm. Tensile strength (TS, MPa) and elongation at break (EAB, %) were determined from stress-strain curves obtained from force-deformation data.

Color

Film disks with 40 mm diameter were used for color measurement by a Color Difference Meter (WSC-S, Shanghai Precise Scientific Apparatus, Shanghai, China). CIE-Lab coordinates were obtained from the reflection spectra of the samples using a D65 illuminant. The exposed area was sufficiently greater in relation to the illuminated area to avoid any edge effect. The Hunterlab parameters: L, a, and b were measured according to a standard test method (ASTM, 1995b). At least five positions were randomly selected for each sample. Color parameters ranged from black ($L = 0$) to white ($L = 100$); greenness ($-a$) to redness ($+a$); and blueness ($-b$) to yellowness ($+b$).

Table 1. Properties of Ch-Ku-As film-forming solutions.

Solution	Contact angle (°)[a,b]		Non-Newtonian index (*n*)	Consistency coefficient (*K*)	Correlation coefficient (*R*)
	On glass	On paraffin wax			
Ch-Ku(Aa)-As	27.8 ± 1.2[a]	85.7 ± 1.7[a]	0.8150	0.3128	0.9986
Ch-Ku(La)-As	27.8 ± 2.2[a]	83.4 ± 5.1[a]	0.8337	0.2702	0.9994
Ch-Ku(Ma)-As	27.0 ± 0.4[a]	82.9 ± 1.3[a]	0.9585	0.1127	0.9997

[a] Data were shown in values ± standard deviation (n=6). [b] Different superscript letters in the same column indicated significant differences ($P < 0.05$).

Statistical analysis

Analysis of variance was performed by ANOVA procedures of the SPSS software (version 13.0, Statistical Package for the Social Sciences Inc., Chicago, USA). LSD test was used to determine the difference of means, and $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Contact angle

Contact angle is usually the angle of liquid where a liquid/vapor interface meets a solid surface, and its value is mainly affected by both of the droplet's inherent properties and the liquid-solid interaction. Contact angle of FFS reflects its wetting behavior on food surface, subsequently affects its adhesion ability. In the paper, glass was chosen as hydrophilic solid and paraffin wax was chosen as hydrophobic solid in order to simulate the wetting behaviors of FFSs on different polar solid surfaces. The static contact angles of FFSs were measured by sessile drop technique.

It was found in Table 1 that the contact angle values of FFSs on glass substrates were all small, indicating the solutions were easy to spread on a hydrophilic substrate. When FFSs were dropped on the surfaces of paraffin wax, the hydrophobic groups of the surfactants preferred to interact with wax, whereas the hydrophilic groups tended to access to solutions. That is, the amount of surfactant adsorbed by the solid surface increases with increasing the concentration of the active agent, and consequently the interface tension between solid and liquid is reduced and contact angle decreases. When the concentration reaches or exceeds a critical value, the adsorption amount is no longer increasing, and the interface tension and contact angle hardly change. A literature showed that the critical micelle concentration of tween 20 is less than 0.01% (Garstecki et al., 2005). In this experiment, the concentration of tween 20 was 0.1%, so we could consider that the contact angles of FFSs on paraffin wax surfaces have reached to the lowest values. Besides, the contact angle values of FFSs on paraffin wax substrate were all less than 90°, lower than those of normal starch FFSs (Xu et al., 2005).

However, the contact angle values did not show significant differences among FFSs prepared by three acids ($P > 0.05$), which indicated that acid types did not obviously affect the wettability of FFSs.

Rheological measurement

The rheological curves of FFSs were shown in Figure 1. The results displayed that, at the same shearing rate, the sequence of apparent viscosity was Ch-Ku(Aa)-As ≥ Ch-Ku(La)-As > Ch-Ku(Ma)-As. Apparent viscosity of a polymer solution at a given shearing rate is mainly determined by the free volume of the fluid and the entanglements among macromolecules. The free volume refers to the space not occupied by atoms, where the diffusion of macromolecular chain segments mainly occurs. If the free volume decreases, the space of molecular motion will reduce, and thus the viscosity of polymer fluid will increase. Moreover, certain numbers of intermolecular-entanglements are retained after the dissolution of polymer. Greater molecular weight and longer molecular chain will result in greater density of the entangled points, higher resistance of the molecular movement, and thus larger viscosity of polymer fluid. According to previous research, chitosan forms more dimers in acetic acid solution than in other acid solutions, which suggests more compact structures of FFS than those prepared with other acid solutions (Park et al., 2002).

From Figure 1, we can also find that FFSs belong to pseudoplastic fluid. The apparent viscosity of Ch-Ku(Aa)-As and Ch-Ku(La)-As dropped substantially with the increase of shearing rate, while the change range of Ch-Ku(Ma)-As was small at different shear rates.

The smaller changes of viscosity for Ch-Ku(Ma)-As may be attributed to the strong acidity of malic acid, which produced smaller numbers of macromolecules tangles. Thereupon, shear rate had little effect on its apparent viscosity. In
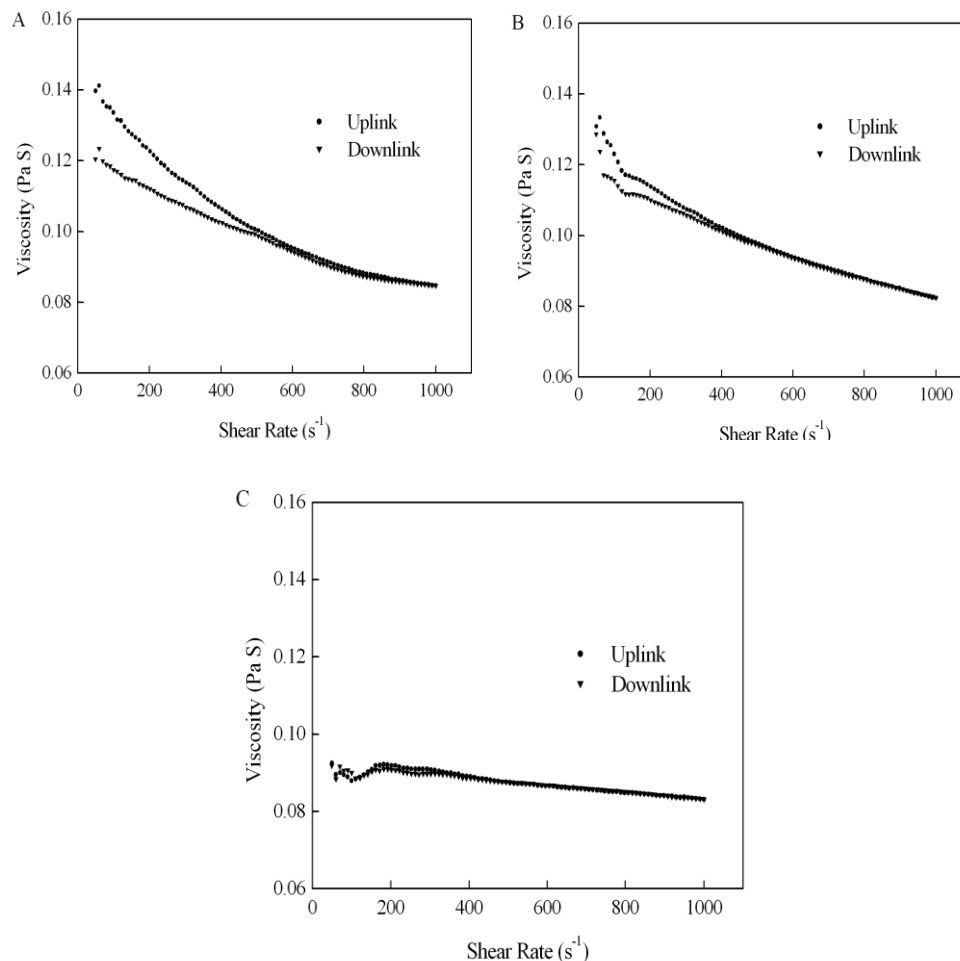
Figure 1. Rheological curves of Ch-Ku-As film-forming solutions (A: Ch-Ku(Aa)-As film–forming solution, B: Ch-Ku(La)-As film–forming solution, C: Ch-Ku(Ma)-As film–forming solution).

addition, the lag area of Ch-Ku(Aa)-As and Ch-Ku(La)-As was relatively similar, and the Ch-Ku(Ma)-As hardly showed hysteresis loop. This phenomenon indicated that when the solutions were subjected to shear, Ch-Ku(Ma)-As was more likely to return to the initial state compared with the other two FFSs.

We can calculate the parameters of rheological properties by fitting lnγ versus lnτ. As observed in Table 1, the non-Newtonian indexes of Ch-Ku(Aa)-As, Ch-Ku(La)-As and Ch-Ku(Ma)-As were 0.8150, 0.8337, and 0.9585, respectively. Results showed that the pseudoplastic decreased, Newtonian increased, and consistency reduced following the sequence of Ch-Ku(Aa)-As, Ch-Ku(La)-As and Ch-Ku(Ma)-As.

Antibacterial tests

Results of antibacterial experiment were presented in Figure 2. As for *E. coli*, it was quite clear that OD values of the control assay increased consistently during the test

indicated an appreciable growth of the bacteria, but the OD values were suppressed greatly in suspensions containing Ch-Ku-As films.

After treated with Ch-Ku(Aa)-As film for 30 min, the OD values of the bacteria suspensions were significantly lower than in the control group ($P < 0.05$). However, 15 min after adding Ch-Ku(La)-As and Ch-Ku(Ma)-As films, the growth of *E. coli* can be effectively inhibited. Figure 2B showed that *S. aureus* kept growing in each experimental group throughout the whole stage, but the Ch-Ku-As films significantly inhibited ($P < 0.05$) the growth trends of bacteria.

Ascorbic acid and surfactant can improve the antibacterial ability of edible packaging film (Bastos et al., 2009). For ascorbic acid, on the one hand it can enhance the acidity of the solution and deteriorate the bacterial growth environment; on the other hand, the rapid reaction between ascorbic acid and oxygen hinders the oxygen source for aerobic bacteria (Tajkarimi and Ibrahim, 2011).

The reason why surfactant enhances antibacterial ability of the composite films is mainly due to its special
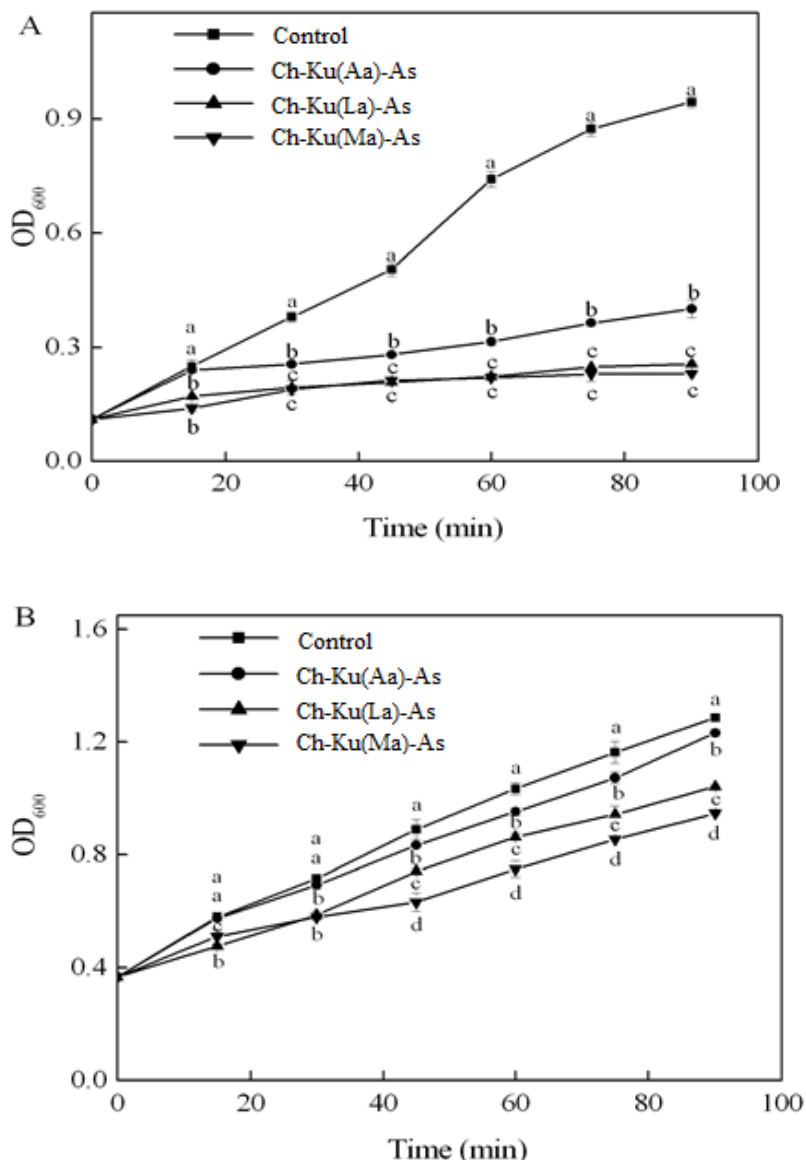
Figure 2. Growth curves of bacteria in the presence of Ch-Ku-As films (A: *E. coli*, B: *S. aureus*).

molecular structure. The format of chitosan in acidic solution is usually the aggregation of several molecular chains. When surfactant is added, the amino or hydroxyl groups of chitosan can interact with the hydrophilic groups of the surfactant, forming dynamic association bodies; at the meantime, long hydrophobic alkyl-chains can induce chitosan to depolymerized into single strands and stretch the chain conformations. Compared with the aggregates, although the numbers of total amino groups did not change, more protonated amino groups were exposed when chitosan in single chain format. Consequently, the numbers of positively charged amino groups in contact with the bacterial cells increased. In addition, the long alkyl-chains of surfactant tended to

infiltrate into the internal hydrophobic membranes of the bacteria due to the hydrophobic interaction, making the interactions between chitosan and bacterial cells more easily, and more effectively interrupting the normal functions of the bacteria membranes.

Release test

According to the release data, the release process of ascorbic acid was generally divided into three stages (Figure 3):

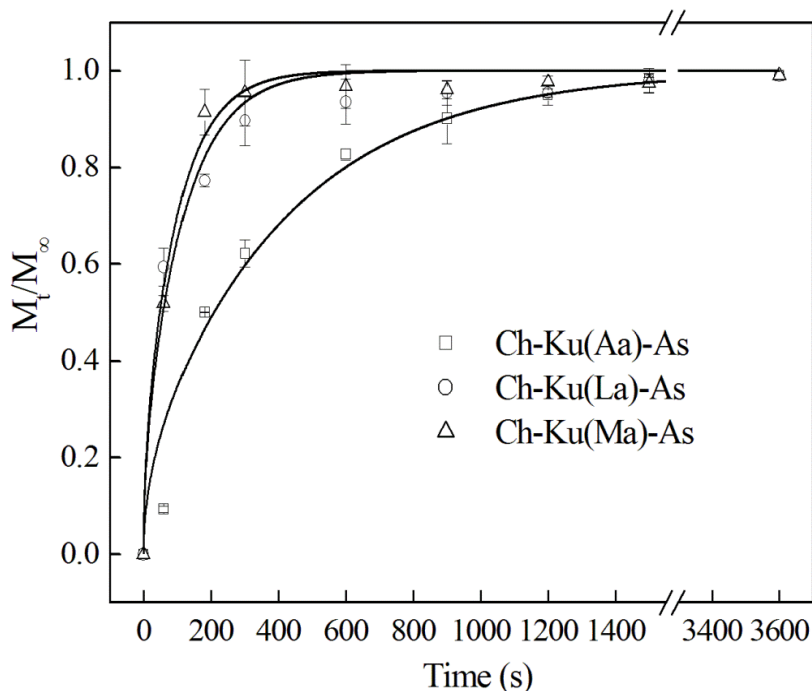1) Rapid release stage: In this stage, about 50 to 90% of

Figure 3. Release behaviors of ascorbic acid from Ch-Ku-As films.

the total ascorbic acid fast released to water, caused by the rapid release of the ascorbic acid adsorbed on the film surface,

2) Slow release stage: The amount of released ascorbic acid accounted for 5 to 30% of the total content, which attribute to its slow release from the inside of the composite film,

3) Balance release stage: In this stage, the concentration of ascorbic acid changed very little.

Table 2 presented that the diffusion coefficient (D) of ascorbic acid from Ch-Ku(Aa)-As film was 0.24×10^{-11} m^2 s^{-1}, significantly smaller than the values of the other two films ($P < 0.05$), which was 27.9 and 23.5% for Ch-Ku(La)-As and Ch-Ku(Ma)-As film, respectively.

As for Ch-Ku(Aa)-As film, ascorbic acid release was mainly controlled by the film swelling process. At initial stage, the ascorbic acid molecules in direct contact with water were quickly dissolved. Subsequently, water molecule penetrated into Ch-Ku(Aa)-As network and ascorbic acid gradually diffused into water through the channel formed by swelling. As time goes by, Ch-Ku(Aa)-As film achieved maximum swelling degree and the release of ascorbic acid achieved a dynamic balancing process.

Compared with Ch-Ku(Aa)-As film, Ch-Ku(La)-As and Ch-Ku(Ma)-As films had weak sustained-release effect on ascorbic acid, and the diffusion badly followed Fick's second law. The reason may be that during the test, film erosion kept going for these two films and the network

structures of the films were destroyed. Consequently, ascorbic acid from inner films could be quickly released to water, causing faster release speed.

Water vapor permeability and mechanical properties

Mechanical property reflects the film's ability to protect the physical integrity of foods (Martins et al., 2012). Table 2 showed that Ch-Ku(Aa)-As had the strongest mechanical strength, the average tensile strength was 5.73 MPa. Ch-Ku(La)-As film had the best flexibility, its average elongation at break was 71.51%. The mechanical properties of Ch-Ku(Ma)-As films were between those of the former films. It has been reported, the molecular structures of chitosan dissolved in different solvents varied due to different peculiarities of acid solutions, which in turn influenced the mechanical properties of the resulting films. Similar phenomena were also found by other researchers (Kim et al., 2006).

The water vapor permeability coefficients of Ch-Ku-As films were presented in Table 2. In this paper, the average WVP coefficients of Ku-Ch(Ma)-As film was ca. 27% lower than that of Ku-Ch(Aa)-As film and ca. 40% lower than that of Ku-Ch(La)-As film. The sequences of WVP coefficients were consistent with those in surface wettability test. Adding ascorbic acid could enhance the hydrophilic nature of edible packaging film (Bastos et al., 2009), whereas the hydrophobic interactions between the surfactant (tween 20) and the film -forming   components

Table 2. Diffusion coefficients (D), mechanical properties and WVP values of Ch-Ku-As films [a,b].

Film	TS (MPa)	EAB (%)	WVP (×10^{-11}gm^{-1}s^{-1} Pa^{-1})	D (×10^{-11}m^{2}s^{-1})
Ch-Ku(Aa)-As	5.73 ± 0.81[a]	58.07 ± 5.60[c]	6.64 ± 0.46[b]	0.24 ± 0.01[b]
Ch-Ku(La)-As	2.53 ± 0.26[c]	71.51 ± 4.37[a]	8.10 ± 0.68[a]	0.86 ± 0.11[a]
Ch-Ku(Ma)-As	3.60 ± 0.39[b]	65.52 ± 2.31[b]	4.82 ± 0.40[c]	1.02 ± 0.11[a]

[a] Results of TS, EAB, and WVP are the mean values ± standard deviation (n=8); Results of diffusion coefficient are the mean values ± standard deviation (for TS, EAB, and WVP, n=8; for D, n=3). [b] Different letters in the same column indicate significant differences ($P < 0.05$).

Table 3. Color analysis of Ch-Ku-As films [a,b].

Film	L	a	b
Ch-Ku(Aa)-As	66.52 ± 0.45[a]	19.33 ± 0.54[b]	50.66 ± 0.81[a]
Ch-Ku(La)-As	62.17 ± 0.39[c]	22.58 ± 0.46[a]	52.12 ± 1.32[a]
Ch-Ku(Ma)-As	64.83 ± 0.34[b]	18.44 ±1.24[b]	48.77 ± 1.01[b]

[a] Results are the mean values ± standard deviation (n=8). [b] Different letters in the same column indicate significant differences ($P < 0.05$).

helped to produce better water blocking performance, which resulted in lower WVP values of Ch-Ku-As films than those of other chitosan-starch composite films (Xu et al., 2013).

Color

The color parameters of Ch-Ku-As film were shown in Table 3. The color values of Ch-Ku-As films were lower than general chitosan-starch composite films represented by lower L values, and higher a and b parameters (Choi et al., 2002). Literature described that, the browning of ascorbic acid could easily occur in atmosphere due to its oxidization, and at meanwhile, it could react with free amino acids in chitosan to produce red and yellow pigments (Choi et al., 2002). From Table 3, we also found that ascorbic acid had the most significant influence on Ch-Ku(La)-As film, leading to the minimum brightness and the maximum redness and yellowness.

Conclusions

According to the results obtained in this study, chitosan and kudzu starch could be readily blended to form new edible biocomposite films. The FFSs prepared by three acid solvents belonged to pseudoplastic fluids. According to the sequence of acetic, lactic and malic acid, the pseudoplastic of FFSs reduced, Newtonian behavior enhanced, and consistency increased. The wettability of FFSs on glass substrate was better than that on paraffin wax substrate and not obviously effected by acid solvents. The composite films had significant inhibitory action on the growth of *E. coli* and *S. aureus*, especially

for Ch-Ku(Ma)-As films. In addition, the release of ascorbic acid was effectively delayed by composite films. The release of acetic acid from Ch-Ku(Aa)-As film was mainly influenced by swelling action, and in other two films were controlled by the film corrosion as well. Among the three composite films, Ch-Ku(Aa)-As film had the strongest mechanical strength, Ch-Ku(La)-As film showed the best flexibility, and Ch-Ku (Ma)-As film possessed the strongest antibacterial and water vapor barrier ability. The results suggested that this kind of composite films had potentially application in food industry.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

REFERENCES

Abugoch L, Tapia C, Villamán M, Yazdani-Pedram M, Díaz-Dosque M (2011). Characterization of quinoa proteinechitosan blend edible films. Food Hydrocolloid. 25:879–886. http://dx.doi.org/10.101 6/j.foodhyd.2010.08.008

ASTM (1995a). E96-95 Standard test methods for water vapor transmission of materials. Standard Designations: Annual book of

American society for testing materials, West Conshohocken, PA, USA.

ASTM (1995b). E1925 Standard test method for yellowness index of plastics. Philadelphia: American Society for Testing and Materials.

Averous L, Fringant C, Moro L (2001). Starch-based biodegradable materials suitable for thermoforming packaging. Starch/Staerke, 53:368–371.

Avila-Sosa R, Palou E, Jiménez-Munguía MT, Nevárez-Moorillón GV, Navarro-Cruz AR, López-Malo A (2012). Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films. Int. J. Food Microbiol. 153:66–72. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.10.017 PMid:22100789

Bajpai SK, Chand N, Chaurasia V (2010). Investigation of water vapor permeability and antimicrobial property of Zinc Oxide nanoparticles-loaded chitosan-based edible film. J. Appl. Polym. Sci. 115:674–683.http://dx.doi.org/10.1002/app.30550

Bastos DDS, Araújo KGDL, Leão MHDR (2009). Ascorbic acid retaining using a new calcium alginate-Capsul based edible film. J. Microencapsul. 26:97–103. http://dx.doi.org/10.1080/02652040802175805PMid:18608803

Bergo PVA, Carvalho RA, Sobral PJA, Dos Santos RMC, Da Silva FBR, Prison JM (2008). Physical properties of edible films based on cassava starch as affected by the plasticizer concentration. Packag. Technol. Sci. 21:85–89. http://dx.doi.org/10.1002/pts.781

Bertuzzi MA, Castro Vidaurre EF, Armada M, Gottifredi JC (2007). Water vapor permeability of edible starch based films. J. Food Eng. 80:972–978.http://dx.doi.org/10.1016/j.jfoodeng.2006.07.016

Bonilla J, Atarés L, Vargas M, Chiralt A (2012). Effect of essential oils and homogenization conditions on properties of chitosan-based films. Food Hydrocolloid. 26:9–16. http://dx.doi.org/10.1016/j.foodhyd.2011.03.015

Bourtoom T, Chinnan MS (2008). Preparation and properties of rice starch-chitosan blend biodegradable film. LWT - Food Sci. Technol. 41:1633–1641.

Chen CH, Kuo WS, Lai LS (2010). Water barrier and physical properties of starch/decolorized hsian-tsao leaf gum films: Impact of surfactant lamination. Food Hydrocolloid. 24:200–207. http://dx.doi.org/10.1016/j.foodhyd.2009.09.006

Chillo S, Flores S, Mastromatteo M, Conte A, Gerschenson L, Del Nobile MA (2008). Influence of glycerol and chitosan on tapioca starch-based edible film properties. J. Food Eng. 88:159–168. http://dx.doi.org/10.1016/j.jfoodeng.2008.02.002

Choi MH, Kim GH, Lee HS (2002). Effects of ascorbic acid retention on juice color and pigment stability in blood orange (Citrus sinensis) juice during refrigerated storage. Food Res. Int. 35:753–759.http://dx.doi.org/10.1016/S0963-9969(02)00071-6

Devlieghere F, Vermeulen A, Debevere J (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol. 21:703–714. http://dx.doi.org/10.1016/j.fm.2004.02.008

Fatih YG, Yener FY, Yemenicioglu A (2009). Antimicrobial activity of lactoperoxidase system incorporated into cross-linked alginate films. J. Food Sci. 74:73–79. http://dx.doi.org/10.1111/j.1750-3841.2009.01057.xPMid:19323761

Fernandez-Saiz P, Lagaron JM, Ocio MJ (2009). Optimization of the biocide properties of chitosan for its application in the design of active films of interest in the food area. Food Hydrocolloid. 23:913–921. http://dx.doi.org/10.1016/j.foodhyd.2008.06.001

Flores S, Haedo AS, Campos C, Gerschenson L (2007). Antimicrobial performance of sorbates supported in a tapioca starch edible film. Eur. Food Res. Technol. 225:375–384. http://dx.doi.org/10.1007/s00217-006-0427-5

Garstecki P, Fuerstman MJ, Whitesides GM (2005). Nonlinear dynamics of a flow-focusing bubble generator: An inverted dripping faucet. Phys. Rev. Lett. 94:1–4. http://dx.doi.org/10.1103/PhysRevLett.94.234502

Gerschenson L, Goyanes S (2009). Starch–vegetable fibre composites to protect food products. Carbohyd. Polym. 75:230–235. http://dx.doi.org/10.1016/j.carbpol.2008.06.018

Kim KW, Min BJ, Kim YT, Kimmel RM, Cooksey K, Park SI (2011). Antimicrobial activity against foodborne pathogens of chitosan

biopolymer films of different molecular weights. LWT-Food Sci. Technol. 44: 565–569.

Kim KM, Son JH, Kim SK, Weller CL, Hanna MA (2006). Properties of chitosan films as a function of pH and solvent type. J. Food Sci. 71:E119–E124. http://dx.doi.org/10.1111/j.1365-2621.2006.tb15624.x

Martins JT, Cerqueira MA, Vicente AA (2012). Influence of a-tocopherol on physicochemical properties of chitosan-based films. Food Hydrocolloid. 27:220–227. http://dx.doi.org/10.1016/j.foodhyd.2011.06.011

Mastromatteo M, Barbuzzi G, Conteb A, Del Nobile MA (2009). Controlled release of thymol from zein based film. Innov. Food Sci. Emerg. Technol. 10:222–227.http://dx.doi.org/10.1016/j.ifset.2008.11.010

Nisperos-Carriedo MO (1994). Edible coating and film based on polysaccharides. In Krochta JM, Baldwin EA, Nisperos-Carriedo MO, (Eds.), Edible coatings and films to improve food quality. Lancaster: Technomic Pub. Co. Inc.

Park SY, Marsh KS, Rhim JW (2002). Characteristics of different molecular weight chitosan films affected by the type of organic solvents. J. Food Sci. 67:194–197. http://dx.doi.org/10.1111/j.1365-2621.2002.tb11382.x

Peressini D, Bravin B, Lapasin R, Rizzotti C, Sensidoni A (2003). Starch–methylcellulose based edible films: rheological properties of film-forming dispersions. J. Food Eng. 59:25–32. http://dx.doi.org/10.1016/S0260-8774(02)00426-0

Pitak N, Rakshit SK (2011). Physical and antimicrobial properties of banana flour/chitosan biodegradable and self sealing films used for preserving Fresh-cut vegetables. LWT-Food Sci. Technol. 44:2310–2315.

Siracusa A, Rocculi K, Romani P, Rosa M (2008). Biodegradable polymers for food packaging: A review. Trends Food Sci. Technol. 19:634–643. http://dx.doi.org/10.1016/j.tifs.2008.07.003

Skurtys O, Dieulot JY (2013) . Classification, modeling and prediction of the mechanical behavior of starch-based films. J. Food Eng. 119:188–195. http://dx.doi.org/10.1016/j.jfoodeng.2013.05.028

Tajkarimi M, Ibrahim SA (2011). Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on Escherichia coli O157:H7 in laboratory medium and carrot juice. Food Control. 22:801–804. http://dx.doi.org/10.1016/j.foodcont.2010.11.030

Talja RA, Heleïn H, Roos YH, Jouppila K (2007). Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. Carbohyd. Polym. 67:288–295. http://dx.doi.org/10.1016/j.carbpol.2006.05.019

Vásconez MB, Flores SK, Campos CA, Alvarado J, Gerschenson LN (2009). Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings. Food Res. Int. 42:762–769. http://dx.doi.org/10.1016/j.foodres.2009.02.026

Woggum T, Sirivongpaisal P, Wittaya T (2014). Properties and characteristics of dual-modified rice starch based biodegradable films. Int. J. Biol. Macromol. 67:490–502. http://dx.doi.org/10.1016/j.ijbiomac.2014.03.029 PMid:24680811

Xiao Q, Tong QY, Lim LT (2012). Pullulan-sodium alginate based edible films: Rheological properties of film forming solutions. Carbohyd. Polym. 87:1689–1695.
http://dx.doi.org/10.1016/j.carbpol.2011.09.077

Xu YX, Kim KM, Hanna MA, Nag D (2005). Chitosan–starch composite film: preparation and characterization. Ind. Crops Prod. 21(2):185–192. http://dx.doi.org/10.1016/j.indcrop.2004.03.002

Review

Resource use conflict in West Africa: Developing a framework for resilience building among farmers and pastoralists

Anthonty N. Onyekuru* and Rob Marchant

York Institute for Tropical Ecosystem Dynamics (KITE) Environment Department, University of York, York UK.

Resource scarcity and security are interconnected. The impact of recurrent resource degradation in West Africa is assessed, in conjunction with social and economic factors, showing how these have interacted with conflict in West Africa and its import in other conflict climes. Resource scarcity interacting with economic pressure and political instability, have resulted in the rapid loss of arable lands in the Sahelian region of West Africa, leading to social crisis across the region. Combined, these factors result in increased land use and social pressure and resultant ownership struggles, which generate conflicts in the southern Sahel. Evidences of resource scarcity and resource use conflict across the region are reviewed and analyzed. These interactions are used to develop a resource use conflict pathway model for building resilience among stakeholders in the region. It is suggested that by making more arable land available through land restoration, in combination with implementing poverty alleviation programmes for the poor, more sustained solutions to the socio-economic and resource crisis in West Africa and across the world could be achieved.

Key words: Climate change, desertification, economic pressure, land restoration, migration, model, ownership struggle, poverty, resource degradation, security, vulnerability.

INTRODUCTION

According to United Nations Economic Commission for Africa (UNECA) (2008), widespread poverty, dependency on climate-sensitive rain-fed agriculture, poor infrastructure, high dependency on natural resources in combination with conflict render West Africa vulnerable to impacts of drought and desertification. Land degradation and desertification are a major cause of forced human migration that can result in conflict, particularly where natural resources are scarce. Ensuing food insecurity, habitat destruction, socio-economic instability, poverty and feedbacks to enhanced climatic variability through land cover change can occur.

Experience have shown that the more people are dependent on sensitive forms of natural capital, and the less they rely on economic or social forms of capital, the more at risk they are from climate change and thus the more likely that they will be provoked to conflict when such resources are threatened (Barnett and Adger, 2007). Stern (2006) referred to how conflict may arise under certain circumstances, mainly as a result of forced migration, which is suggested to affect up to 200 million people globally by 2050. Blench (2004) asserts

that the pressure on arable land in the semi-arid zone increased, soil fertility inevitably decreases making farmers to move to regions of uncleared bush or to increase the area of their land holding, excluding the mobile pastoralists who traditionally treated uncultivated bush as common resource. It is these interconnections that this paper assesses within the context of West Africa. The paper reviews evidence for climate change impacts on resource use and social interactions in the context of the West African Sahelian region. Interconnections are used to develop a conceptual framework (model) that can be used to understand the interactions between climate change, anthropogenic variables and resource use conflicts in Nigeria and West African sub region. The paper answers the certain critical questions: What are the likely resource use conflicts situations in West Africa? What are the predisposing variables for resource use conflicts in West Africa? How are the variables interacting to cause conflict? What are the adaptation pathways that lead to vulnerability and which pathways build resilience to conflict? How are these interactions linked to build a conflict management framework in West Africa and around the world? Answering these questions will offer useful insight to the social, economic, environmental and biophysical linkages and how different intervening variables can lead to different resilience outcomes in natural resource conflict management process, especially in environmental resource dependent and developing economies. Thus, offering useful tools to conflict practitioners, policy makers and social and political scientist in natural resource conflict management across the world.

The paper adopts the method of extensive literature review on the different areas relevant to resource use conflict process and useful in building a framework for conflict management especially among farmers and pastoralists across scale. These information are used to develop a resource use conflict management framework for natural resource dependent systems, thereby providing a veritable instrument in the hands of practitioners for present and future conflict management across the globe.

CONCEPTUAL AND THEORETICAL FRAMEWORK

Here, we present some of the conceptual issues and theoretical foundation relevant to the development of a resource use - conflict framework for conflict management in West Africa and other environmental resource dependent economies.

Conceptual framework

Resource scarcity

Resource scarcity refers to a situation where renewable resources are degraded or decreased or a situation of inequitable distribution of resources within a country or region (UN Interagency Framework Team for Preventive Action (UNIFTPA, 2012). Gendron and Hoffman (2009) opine that resource scarcity can be conceptualized in three ways; the basic human security, if the human population cannot meet its basic dietary requirements. This is also referred to as the minimalist approach; the absolute minimum of resources required to sustain human life. A second interpretation can be defined as current resource availability to meet rising or projected increased demand. A resource in this scenario is considered scarce if there are insufficient resources to meet projected demands. The maximalist approach considers both human and non-human demands on a particular resource (Matthew, 2008). Resource scarcity can also be conceptualized as one of three structural components:

(i) Supply induced scarcity, in which environmental degradation occurs;
(ii) Demand induced scarcity in which there is increased consumption of a commodity; or
(iii) A structural scarcity in which infrastructure and distribution mechanisms unevenly redistribute the resource in question or access is restricted (Homer-Dixon, 1994,1999; Kameri-Mbote, 2004; UNIFTPA, 2012).

Conflict

Several scholars have offered different definitions of conflict: in terms of status, Park and Burges defined it simply as the struggle for status (Bartos and Wahr, 2002). Mack and Snyder (1973) and Himes (1980) defined it as the struggle for scarce resources and significant social change. In terms of struggle for scarce resources; it has been generally seen as a situation in which two or more parties strive to acquire the same scarce resources at the same time (Wellesteen, 2002) defined conflict as a struggle over values and claims to scarce status, power and resources in which the aims of the opponents are to neutralize, injure, or eliminate their rivals. Bartos and Wahr (2002) define conflict as stemming from the desire to achieve incompatible goals and / or to express their hostilities; it is the pursuit of contrary or seemingly incompatible interests – whether between individuals, groups or countries (DFID, 2006). Conflict arises from the interaction of individuals who have partly incompatible ends, in which the ability of one actor to gain his ends depends on an important degree on the choices or decision another actor will take (Olufemi and Samson, 2012). For the purpose of this work we will define conflict simply as a (constructive or destructive) disagreement between groups, arising from incompatible goals on the mode of allocation of (usually

but not limited to common) resources.

Theoretical framework

Conflict theory

The basic insight in conflict theory is that human beings are sociable but conflict-prone animals. Life is basically a struggle for status in which no one can afford to be oblivious to the power of others around him and individuals' behavior is explained in terms of their self-interests in a material world of threat and violence (Collins, 1974). Conflict theory looks at society as being made up of individuals or groups who must compete for social, political, economic and material resources (Figure 1). Marxists argue that economic inequality is at the heart of all societies. Thus, in basic terms, some people will have more than their fair share of society's economic resources and other will consequently have less than their fair share. Collins (1974) looks at people as animals maneuvering for advantage, susceptible to emotional appeals, but steering a self-interested course toward satisfactions and away from dissatisfactions and each individual is basically pursuing his own interests and there are many situations, notably ones where power is involved, in which those interests are inherently antagonistic and the dominant party take advantage of the situation. This need not involve conscious calculation, but a basic propensity of feeling one's way toward the areas of greatest immediate reward, like flowers turning to the light (Collins, 1974). Collier conclude that there is conflict because violent coercion is always a potential resource, and it is zero-sum sort, any use of coercion, even by a small minority, calls forth conflict in the form of antagonism to being dominated. In summary, every individual tries to maximize his subjective status according to the resources available to him and to his rivals.

Specifically, Hardin's *tragedy of the common theory* (Hardin, 1968, 1988) holds that indigenous common land tenure system in Africa encourages the degradation of the resource as a result of many individuals using scarce resource. Homer-Dixon (1991, 1994, 1995, 1999) *environmental scarcity theory* links tension between parties as resulting from the growing vulnerability and insecurity of their livelihood, he argues that environmental change, population growth and unequal social distribution are the three main source of scarcity that lead to conflict (Homer-Dixon, 1994).

Rationale for conflict

The main reasons why conflict exists in resource distribution are:

(i) People believe they are treated unjustly,

(ii) People do not have enough to live a decent life (absolute deprivation) or
(iii) People may have belligerent culture (Bartos and Wahr, 2002).

Absolute deprivation occurs when a party is deprived of whatever it needs to lead a decent life, leading to a sense of frustration. Dollard et al. (1939) opine that frustration is a free floating hostility that can target almost anything at any time. People feel frustrated and get hostile whenever they are prevented from reaching their goals. They argue that whenever individuals get frustrated and are not able to vent it through aggressive actions, the feeling of frustration continues and become very intense that they can attack any person or group that is handy even if it is not the source of their frustration. So, coercion and the ability to "force" others to behave a certain way are the primary basis of conflict (Lepird et al., 2012). Therefore, the basic principle of conflict theory is that the natural evolution of societies is described as a series of clashes between conflicting ideas and forces that at the end of each clash, a new and improved set of idea emerges; change needs conflict in order to be facilitated. This is known as the dialectical process (Olufemi and Samson, 2012).

Causes of conflict

According to Newton's physical theory of motion, each action produces a reaction, same is the case in the social theory of conflict as is depicted in Kant (1724 – 1804) and Hegel (1770 – 1831) theory, that every individual, group, organization or unit in society represent a force whose action stimulates many counter forces, and when forces meet counter forces it either stimulate cooperation or conflict depending on many factors (Bartos and Wahr, 2002). There is ample evidence to show that scarcity (crop failure, common wells, common lands) has always been an elementary and ever present conditions of existence in human history which leads to conflicts and wars (Baechler, 1999; Hilyard, 1999). Hence, Shetima and Tar (2008) opine that conflict over scarce environmental resources form an intrinsic part of dialectical interaction between human beings and nature and has been endemic in those areas where the environment, economic and social conditions have combined to predispose the two groups to a competitive encounter (Figure 1).

However, some post-modern scholars question the notion of scarcity and argue that it is a matter of human definition and man-made phenomenon, connected to factors such as power, distribution, drawing of boundaries and international politics. Even though there may not be a cause – effect relationship between conflict of interest in resource use, competition and conflict, Shetima and Tar (2008) opine that the likelihood is increased with scarcity
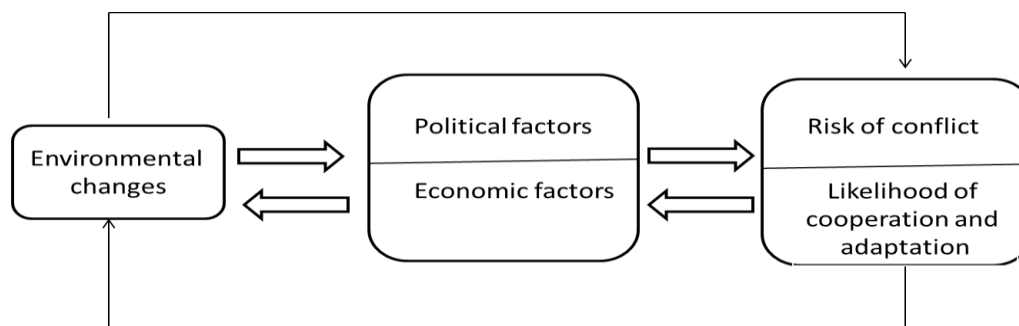
Figure 1. Indirect effects of environmental changes on conflict and cooperation Source: Bernauer et al. (2011).

of resources upon which the groups depend. Thus, our recognition of socially generated conflict (insufficient necessities for some people and not for others) is not to deny absolute scarcity (insufficient resources), no matter how equitable they are distributed (Hilyard, 1999).

In the case of Nigeria conflict arises between pastoralists and farmers as a result of early southward movement of herders in the sedentary zones before the harvest is complete. And this farmer – pastoralists conflict is mostly acute around the semi-arid zones due to the frequency of shortage of rains (Scoons, 1995). In Mali's Niger River delta, conflict between the farmers and the herder occur whenever the delta is drier and the farmers are forced to cultivate deeper parts of the delta, encroaching into the grazing areas, which angers the patoralist (Moorhead, 1989). In Nigeria, due to shifts in planting techniques driven by changing climate, many farmers now plant over designated grazing routes long agreed upon with herders, leading to violence (Sayne, 2011).

The conflict process

Four important conditions influence the likelihood that resources will be the object of conflict.

(i) The degree of scarcity;
(ii) The extent to which the supply is shared by two or more groups;
(iii) The relative power of those groups; and
(iv) The ease of access to alternative sources (Ehrlich et al., 2000)

So depending on what the situation is and the mix of variables, the nature of conflict and the resilience of the people will vary. According to Tamas (2003), conflict is generated by the scarcity of natural resources in two primary ways. The first mechanism is that resource scarcity drives elite to "capture" resources, marginalizing powerless groups in the process. The second way scarcity supposedly causes conflict is through its

debilitating effect on economic and social innovation – the "ingenuity gap" (Homer-Dixon, 1999), in which case the society is unable to utilize resources due to their lack of technical-know-how to exploit the resource. In their analysis of resource use conflict process, Ehrlich et al. (2000) opine that scarcities of renewable resources (such as cropland, fresh water and forests), due to their increased demand and/or their unequal distribution lead to their degradation and depletion and thus produce civil violence and instability by generating intermediate social effects, such as poverty and migrations, that analysts often interpret as the conflict's immediate causes. As a result powerful groups capture valuable environmental resources ("resource capture") and prompts marginal groups to migrate to ecologically sensitive areas ("ecological marginalization"), which in turn reinforce environmental scarcity and raise the potential for social instability.

Societies can adapt to environmental scarcity either by using their indigenous environmental resources more efficiently or by decoupling from their dependence on these resources, depending upon the supply of social and technical "ingenuity" available in the society. If on the other hand adaptation is unsuccessful, environmental scarcity constrains economic development and contributes to migrations, situations which sharpens existing distinctions among social groups, weakens states and in turn makes them vulnerable to ethnic conflicts, insurgencies and coups d'etat (Ehrlich et al., 2000). These two concepts - adaptation and vulnerability are the key cornerstones of our conflict pathway model in this paper (Figure 1).

Resource scarcity and conflict

Several scholars have linked resource scarcity to violent conflict, starting with the work of Thomas Malthus in his Essay on the Principle of Population (1798) and the neo-Malthusianism (Hardin, 1968; Homer-Dixon, 1994; Renner, 1997; Kahl, 2006; Ban, 2007). In his essay, Malthus claimed that the human population grows

exponentially, while the supply of food only grows, at best, in an arithmetic ratio. This was believed to inevitably lead to subsistence crises. In his article, 'The Tragedy of the Commons,' Hardin (1968) picks up the Malthusian thread by using medieval grazing commons as a metaphor for problems of collective behaviour and use of resources (Theisen, 2006). He warns that what is seen as individually rational, in terms of resource consumption, always points in the opposite direction of the public interest. Homer-Dixon (1999) sees population growth as the main cause of scarcity. Neo-Malthusianism agree that there is scarcity of renewable resources that acts as important constraints on human behaviour, and that there are linkages between this and violent conflict. Since these areas are often densely populated, neo-Malthusians argue that this will lead to large-scale migration, which in turn spurs conflict (Homer-Dixon 1999; Renner 1997) (Figure 1). They also argue that environmental degradation is often following a non-linear pattern, making substitutions and preventive measures hard to apply (Homer-Dixon 1999; Kahl 2006). In particular, Homer-Dixon (1994, 1999) argues that decreasing access to renewable resources increases frustration, which in turn creates grievances against the state, weakens the state and civil society and increases the opportunity for instigating an insurrection.

Several studies provide evidence that environmental scarcity is the cause for many recent conflicts. Perhaps Barbier and Homer-Dixon's (1996) efforts to link environmental scarcity and conflict through the inability of resource-poor countries to adapt to economic conditions and pressures offer, however, a clearly testable hypothesis linking resource scarcity to conflict (Tamas, 2003). Another effort to test the proposition is the work of Hauge and Ellingsen (1998), who found a positive effect of environmental degradation on conflict, particularly at lower levels of violence. Also UNEP (2009) found that natural resources play role in 40% of all violent intrastate conflicts. Study by the Toronto Group's Environmental Change and Acute Conflict Project (ECACP) and the Environmental Conflicts Project (ENCOP) at ETH Zurich, found a link between violence in South Africa, the insurgency in Assam, and the Zapatista rebellion in Chiapas (Homer-Dixon, 1991, 1994, 1999; Homer-Dixon and Blitt, 1998; Percival and Homer-Dixon, 1998), Rwandan genocide (André and Platteau, 1998; Bächler, 1999; Ohlsson, 1999), ethnic clashes in Kenya (Kahl, 1998, 2006), the conflict between Israelis and Palestinians (Homer-Dixon, 1994), the civil war in Sudan (Suliman, 1997), as well as the Middle East and Nigeria (Spillmann, 1995; Bächler et al., 1996; Bächler, 1998) and environmental degradation (Theisen, 2006; Bernauer et al., 2011). In their work on rainfall as an instrument for economic shocks in Sub-Saharan economies, Miguel et al. (2004) measure deviations in precipitation and conclude that negative deviation increases the risk of conflict. They conclude that the recruitment cost of rebel soldiers decreases when there is low agricultural output. Lagged

percentage change in rainfall, relative to the previous year, was found to increases the risk of onset of conflicts in Sub-Saharan Africa (Hendrix and Sarah, 2005). Raleigh and Urdal (2005) found a significant relationship between freshwater availability per capita and conflict. Also rainfall deviations above a certain threshold heighten the risk for the outbreak of civil war between 1980 and 2002 (Levy et al., 2005). Of the 37 cases of communal clashes reviewed by Fasona and Omojola (2005) in Nigeria, 19 were triggered by land resource issues. Corroborated by Nyong (2007), who in his study, found that resource use conflict accounts for about 54% of all communal clashes in Nigeria. This is more prevalent when individuals are forced to migrate fron the area of scarcity to other areas of perceived abundance. To this end, Suhrke (1993) contends that whether or not resource scarcity induced migration leads to conflict in receiving areas depends on the capacity of the state to accommodate the needs and alleviate the grievances of the migrants and locals alike. Thus, linking conflict to the socio-politico-economic system (Figure 2).

Theisen (2006) asserts that the most profound arguments for linkages between scarcity and conflict have been put forward by Homer-Dixon (1991, 1994, 1999; Homer-Dixon and Blitt, 1998). He argues that rural-to-rural migration, motivated by scarcity in the place of departure, leads to further ecological and economic decline at the landing spot. This is either because the newcomers do not have sufficient local knowledge to treat the local ecosystems properly and/or that the ecosystem is especially vulnerable, leading to over exploitation and irremediable damage to the renewable resources. This process is labeled environmental marginalization and is argued to have caused deprivation conflicts in Chiapas (Mexico), the Philippines (Homer-Dixon, 1999) and in the Brazilian Amazon (López, 1999).

Overall, the existing evidence suggests that resource scarcity may, under specific circumstances, increase the risk of violent conflict. In most cases as is in agreement within scarcity literature, the conflict potential of scarce resources is most relevant for less developed countries as their dependence on them is greatest (Bächler, 1999; Diamond, 2005; Gleick, 1989; 1993; Homer-Dixon, 1991; 1994; 1999; Homer-Dixon and Blitt, 1998; Kahl, 1998; 2006; Myers, 1993; Ohlsson, 1999, 2003; Petzold-Bradley et al., 2001; Renner, 1997; Suliman, 1993). In poor societies where many people already live on the margin of subsistence, the effect of increasing scarcities will be increasing inequalities leading to factional conflict over government (Theisen, 2006).

CLIMATE CHANGE IMPACTS ON NATURAL RESOURCES IN WEST AFRICA

Evidence have shown that the long-term decline in rainfall from the 19 70s has caused a 25 to 35 km southward shift in the Sahel, Sudan and Guinean
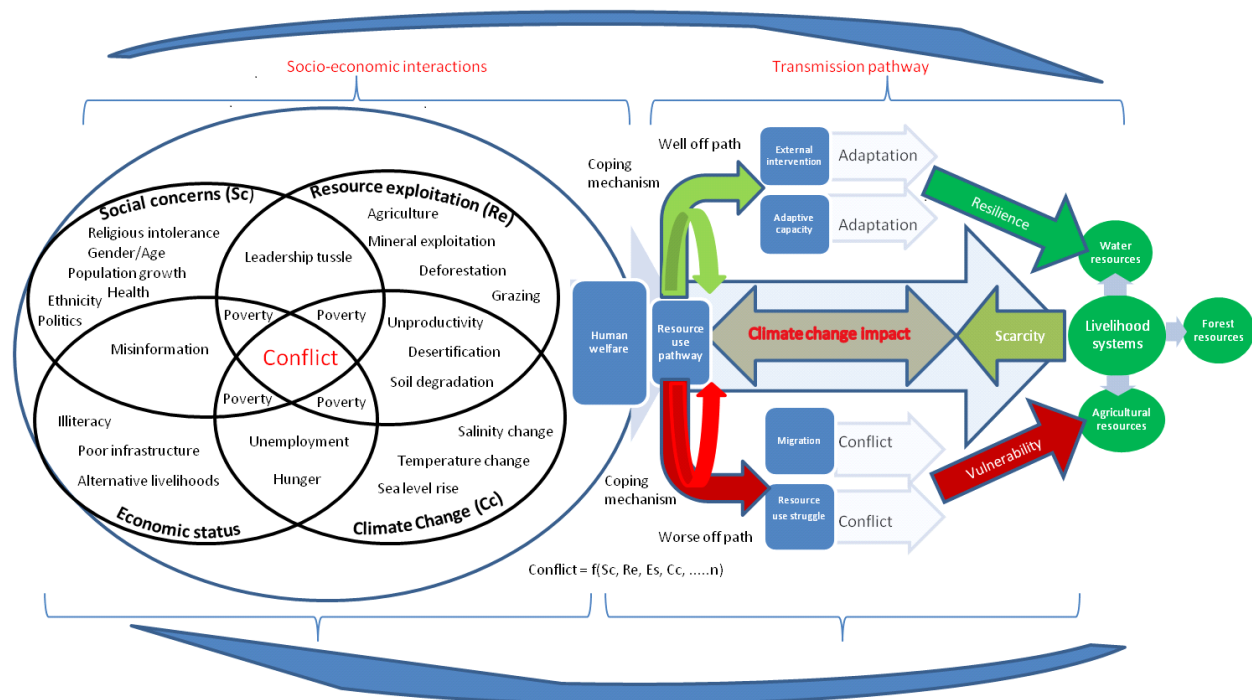
Figure 2. Climate change – conflict pathway model for a natural resource dependent system.

ecological zones of West Africa (Gonzalez, 2001); resulting in the loss of grassland / acacia woodlands and shifting sand dunes in the Sahel (ECF and Potsdam Institute, 2004). In Nigeria for example there was a 425% increase in the extent of sand dunes/aeolian deposits between 1976 and 1995 around the northern axis of the country; desert now covers about 35% of Nigeria's land mass (Fasona and Omojola, 2005); this southerly migration of arid land results in Nigeria losing 3509 km^2 of rangeland and cropland to desertification each year, particularly affecting eleven northern states (Brown, 2006). According to Okpi (2010), about 200 villages in Yobe State, towards the northern border of Nigeria with Niger Republic, have been incorporated within the Sahara desert. In Yobe and Gombe States alone, over 60,000 ha of farmland have been lost to desertification; aerial photographs show that 30,000 ha of productive land have been occupied by the encroaching dunes during the last quarter of the 20th century, removing grazing lands from 30,000 head of cattle year^{-1} (Olori, 2002). Between 50 to 75% of land areas in the northern states (which account for about 43% of Nigeria's land mass) are affected by desertification and are particularly vulnerable to soil degradation (Yusuf, 2009). Yusuf (2007) showed that with a conservative production figure of 100 kg of millet hectare^{-1}yr^{-1}, that the 60,000 ha destroyed by the dunes in Yobe state Nigeria is capable of producing over 60,000 bags of millet; with an average grain requirement of one bag of 100 kg of millet per family of four people per month, this loss would have

been sufficient to sustain 20,000 people per year.

Furthermore the rate of forest conversion is spatially quite variable with some regions in Nigeria, such as the Mandara Plateau mosaic, Cross-Niger transition forests, Jos Plateau forest-grassland mosaic and the lowland forests, having more than a 95% transformation rates (UNECA, 2008). Between 1980 and 2005, up to 3.3% of West African forests were lost to exploitation due to high dependency of national and domestic economies on available natural resources (Atta-Asamoah and Aning, 2011). The implication of a continuous depletion of the sub-region's forest through deforestation is increasing expansion of desertification which has serious implications on the patterns of migration between the Sahel and forest areas of the sub-region (Atta-Asamoah and Aning, 2011). The movement of people southwards has resulted in loss of primary forests and woodlands, repeated logging and clearing of land for agriculture. One of the biggest impacts on the forest has been the extraction of trees for charcoal making (about 150 million tonnes year^{-1} from the savannah and woodland areas), and the use of high-value woods for timber, most affected are *Dalbergia melanoxylon*, *Khaya* spp and *Pterocarpus erinaceus* (UNECA, 2008).

The situation in Nigeria is also reflected in adjacent West African countries, as major urban centres such as Accra, Kano, Niamey, Nouakchott and Ouagadougou are located within areas most affected by the observed changes in climate (UNEP, 2011). In Ghana about one third of the land area is threatened by desertification

(UNEP, 2008) and Ghana's savanna areas have been increasing at an average rate of 1.2% per year from 1972 to 2000 (Idinoba et al., 2010). The main area where resource scarcity could challenge political and economic stability in Ghana is managing the north-south divide where water is needed for the production of energy in the south and to support agriculture in the north; the cocoa production being vital for economic stability. In Niger, about 65% of the territory lies within the Sahara Desert, it is estimated that the desert is expanding by about 200,000 hectares annually (Mongabay, 2006) and it's forests are also shrinking; Niger has lost some third of its forests since 1990 (UNEP 2008). In Burkina Faso desertification is one of the key drivers behind 60% of recent urban population growth (UNCCD, 2004). Longer dry seasons are already driving farmers to migrate from northern and central parts of the country into the fertile east and west, bringing them into contact with settled farmers (Brown and Crowford, 2008).

A report by MECV and SP/CONEDD (2006) shows that drought, land degradation, deforestation and the partition of water between Burkina Faso and Ghana will be a delicate issue in coming years, especially if climate change leads to significantly lower rainfall and run-off. Together Ghana and Burkina Faso share 85% of the Volta basin and much of the Volta's flow travels through Burkina Faso before reaching Ghana (Brown and Crowford, 2008). The Volta contributes 56% of inflows into Dams in Ghana and produce more than 90% of Ghana's electricity (Filho, 2011). Thus, any decline in water level will impact directly on energy production in Ghana. The Senegal River has its main source in the Fouta-Djalon Mountains in Guinea and provides water to the semi-arid parts of Mali, Senegal, and Mauritania. Eight severe drought events have occurred during the period from 1970 to 1980, leading to chronic rainfall deficits. In 1988 and 2000, real crisis led to the consequent loss of lives in both countries (Transboundary Freshwater Dispute Database, 2000). The Niger River basin, spreads over ten countries. Niasse (2007) asserts that there is the risk that water conflict could be blamed on upstream countries for what is really the fault of climatic change. Brooks (2006) suggests that the Tuareg rebellion in Mali 1990 began amid famine and widespread political repression despite being portrayed as an attempt by various Tuareg groups in Niger and Mali to secure an autonomous Tuareg state. Thus, a major impact of climate change in West Africa that is impacting on international relations is that on the trans-boundary water resources.

Lake Chad crosses four countries, with the biggest share located in Chad, then Nigeria, Niger and Cameroon. In 1960 it covered 45,000 km^2 but now only 550 km^2 (Urama and Ozor, 2010; Filho, 2011). Lake Chad provides a lifeline to millions of people living in the catchment area; for sanitation, drinking, agriculture, fishing and religion / cultural activities, further shrinkage

of the lake resulting from climate change will undermine the very base of human development in the basin, including in the north-east zone of Nigeria. Many villages have sprung up in areas where the lake has disappeared; these have been causing tension among the different countries with arguments over territory between various right claimants (Urama and Ozor, 2010). As the water of the lake recedes, farmers move closer to the lake's shoreline to cultivate the emerging lands and pastoralists move closer to the remaining water to feed their livestock, accentuating the rate of contact between major livelihood systems and thus sowing the seed of competition and conflict (Onuoha, 2010). More fundamentally, international boundaries of the lake has been blurred in the region, Nigerians and Nigeriens have crossed political borders in pursuit of the receding waters, as well as the migration of citizens of Chad further south in search of optimum opportunities. These long-distance migrants have been well-armed since the mid-1990s and are willing to use violence to assure their grazing (Blench, 2004). Most fishermen have converted to farming, but this may not be sustainable, as with less rainfall agricultural areas need water to irrigate their crops, and they will continue draining what is left of Lake Chad. The problem is expected to worsen in the coming years as population and irrigation demands continue to increase.

The risk of conflict degenerating into inter-communal clashes in the region could manifest in the near future if existing political institutions fail to reconcile conflicting interests over access to such shared water resources and in a situation where governance institutions concerned are weak, inequitable water management can heighten (Onuoha, 2008a, b). This situation is not just in West Africa, a recent United Nations report reveals that more than 600 lakes in Africa are declining rapidly owing to the combined impact of climate change and resource overuse (UNEP, 2006). Also, water scarcity is already known to afflict 300 million people and claim at least 6 000 lives annually in Africa (Integrated Regional Information Network (IRIN), 2004).

RESOURCE SCARCITY – CONFLICT PATHWAY MODEL; THE THEORETICAL LINK

Having explored on the link between conflict and resource scarcity in the previous sections, it is clear that resource scarcity acts in conjunction with a complex blend of economic, social, political and institutional factors (Figure 1), that eventually breed violence and determine adaptation capacity (Figure 1) (Martin, 2005; Kahl, 2006; Buhaug et al., 2008; Krummenacher, 2008; Salehyan, 2008a; Bernauer et al., 2011; Koubi et al., 2012; UNIFTPA, 2012). These variables in concert with human actions undermine the availability of natural resources, bringing about scarcity and the resultant struggle for the existing ones (conflict) (Figure 1).

Economic and social variables tend to have a much larger effect on conflict and overshadow the effects of the environmental variables (de Soysa, 2000). The Environmental Conflicts Project (ENCOP), found that resource use conflict manifest as political, social, economic, ethnic, religious or territorial conflicts, or conflicts over resources or national interests, or any other type of conflict (Baechler, 1998).

Governance factors influence the range of response options available to different groups; including migration, adaptation strategies, coping and survival strategies, or direct violent conflict (Figure 1) (Bernauer et al., 2011). Adaptation requires response on multiple levels and appropriate interventions depend on the mix of drivers (UNIFTPA, 2012), thus, scarcity does not just cause conflict, but (1) the interaction of the other variables; if positive leads to proper adaptation and if negative leads to conflict and (2) the path which the society chooses to follow can either lead to resilience or vulnerability (Figure 1).

The model

Crisis due to resource scarcity is a daily signature dotting the landscape of West Africa, impacting on human populations, their livelihoods and social connections across the wider West African region. To manage its impacts and reduce resource and social conflicts, we need to identify the interconnections that exist between society, resources and the resource scarcity in what we term the "resource use – conflict pathway model" (Figure 1). The notion of resource use derived conflict has its roots in neo-Malthusian notions of carrying capacity and the interface between human population and available resources. As population increases there will be a scramble for the available resources which deplete with time. The model showed that when a society depends on scarce natural resource, (land, water, forest, agriculture) for their livelihoods, incidence of climate change in conjunction with other anthropogenic variables (Figure 1), will impact on human welfare by creating scarcity. Separately or in combination with other factors resource scarcity can distablize livelihoods, negatively affect ecosystems and undermine peace and development and where local and national institutions lack capacity to resolve that, violent conflict may occur. Depending on the magnitude and direction (+, -) of each of the intervening variables (socio-economic interactions) and the 'transmission pathway', a society achieves peaceful and harmonious coexistence or is ridden with conflict. For example, Collier (2000) found out that a country with large natural resources, many young men and little education is very much more at risk of conflict than one with opposite characteristics, even a slight increase in the level of education can decrease the risk of conflict. Findings also show that the higher the per capita income of the society the lower the risk of conflict (Human Security Report Project, 2007). This is the case in the

West African Sahara area (e.g. northern Nigeria), where there is high amount of young illiterate population. The reverse is the case in southern Nigeria where there is a very high level of youth education.

Operationalizing the model

This model, unlike the other conflict models in literature (e.g Bathos and Wehr, 2002; Bernauer et al., 2011) is unique; more elaborate, robust and operationalizes the linkages among the natural resource - socioeconomic variables and traced their pathways to conflict. Most importantly it x-rays the intra relationships among the actors and what possible outcomes that are expected, and when nipped in the bud does not result in 'poverty' which is the fertile ground for conflict. This is because, the primary cause of conflict is the feeling of deprivation and the quest to make both ends meet at all cost, thus the saying that 'a hungry man is an angry man'. For instance, the advent of climate change (Cc) acts on natural resource base (Re) (agriculture, forestry, water) (Figure 1), to render them unproductive and / infertile or they are over exploited and degraded and the people have no other source of livelihood, poverty sets in. If they have good adaptive capacity (e.g ability to diversify, personal traits) or receive external interventions (from donor agencies and the government as in the case of food aids, help from friends and relatives), then they are better able to adapt and are resilient. Individuals are better able to adapt when they have alternative livelihood options which guarantees their survival. Alternatively, if the individuals struggle with others in the same environment, or migrate to other regions, there will be fierce resistance from the settled communities, *potentially* leading to violent conflicts. This is the major cause of conflict between nomads and farmers across West Africa. While the former pathway results to building resilient societies, the latter perpetually leaves the individuals vulnerable to conflict due to successive displacement of settled communities (Figure 1). In the same vain, the interactions between socio-economic variables (Sc) and natural resources base (Re) results in ownership tussles; who controls the resource. This is the source of most conflicts all over the world. Socio economic variables (Sc) interact with economic concerns (Es) to mis-inform the people about who is responsible for their woes and thus are used by the elites to their peril in the struggle for economic and political powers. Also climate change (Cc) acts to undermine the economic base (Es) of the people in various ramification to create a state of deprivation, hunger and unemployment. In each case the endpoint is poverty and the vicious cycle continues to either breed conflict or build resilient when the right things are done (Figure 1).

In Figure 1 Sc ∩ Re focuses on the struggle for governance and resource control: this is most important in conflict management as different political, religious and

ethnic groups engage each other in the struggle for control. Sc ∩ Es is the socioeconomic dimension of conflict; the former has an overbearing influence on the latter, shaping the livelihoods of the masses with negative nuances and policies, thereby impoverishing the people. Re ∩ Cc concerns the complex biophysical interactions that results in environmental degradation and resource depletion resulting from unsustainable resource exploitation due to 'Sc' and the uncertainties introduced by the exogenous impact of Cc. Es ∩ Cc results from the inability of the people to cope with the impact of Cc due to poverty, lack of knowledge / coping strategy and poor infrastructure, thereby exacerbating an already vulnerable situation. Depending on the prevailing circumstance, the interactions either engender resilience if the individual(s) adopt effective adaptation options or are perpetually vulnerable if they do otherwise.

This analogy is in resonance with those of other conflict scholars. Obioha (2008) asserts that the eco violence theories perspective of conflict explains that conflict is generated by the scarcity of natural resources the product of total *population in the region* and *physical activity per capita*, and second, *the vulnerability of the ecosystem in that region to those particular activities*. A state of deprivation and marginalization results in people becoming highly vulnerable to shock and more ready to join armed groups (de Soysa et al., 1999). Thus, scarcity in itself does not cause conflict, but the ability of the people to cope with scarcity is what determines whether there will be conflict or not. For example, someone who is well off is less likely to be involved in violent conflict. In this respect, the provision of aid, and importantly some certainty that aid will arrive, can help reduce the need for people to use violence to provide for their needs (Gough, 2002; UNIFTPA, 2012). In many developed countries, established and effective welfare systems perform this function, which in part explains why they experience relatively less frequent and intense violent conflicts than developing countries. Human insecurity may in turn lead to more conventional security problems, with an increased propensity for people to engage in violence to protect or develop alternative livelihood strategies. This is a cyclic phenomenon that can be remedied with interventions or adaptation, be it from outside agencies (NGOs, government, international organizations), internal (friends, social groups, family members, community), self-adaptation or a combination of several of these. The complex interaction factors in our model (Figure 1), necessitates individuals to develop resilience through effective adoption of adaptive practices in the system to prevent being perpetually vulnerable to violent confrontation.

SUGGESTED WAYS FORWARD

To develop an effective system for managing conflicts, we must first identify the economic, environmental, social and cultural threats experienced by vulnerable groups. Secondly, we need to understand the causes, characteristics and factors that fuel different conflicts, and how vulnerable households and communities have traditionally managed such conflicts; this information can be used to develop effective conflict management strategies. In doing this three different, but interrelated options are at the disposal of conflict management practitioners; (1) conflict prevention options, (2) conflict remediation options and (3) post conflict management options.

Conflict prevention

Policies aimed at reducing ethnic conflicts and communal clashes in West Africa, must necessarily first link to the restoration of degraded lands, reduction of livelihood vulnerability, promote alternatives and improve the availability and access to natural resources. This should be done in order to mitigate the drivers of migration and conflict and help secure development gains. In the case of our model, it means that stakeholders have to understand the interactions between the different variables and take steps to nip in the bud the negative outcomes of their interaction (degradation, leadership tussle, misinformation, unemployment and hunger) and making sure they are identified in time when they set in, in order to reduce poverty or the feeling of deprivation.

Provision of livelihood options to reduce relative and absolute deprivation so that people can live a pleasant life with their basic needs met is a sure way of reducing vulnerability. There is the need to target transforming key economic, social political and institutional factors that could lead to violent conflicts in the future. This view is also shared by UNIFTPA (2012) that reducing livelihood vulnerabilities and promoting alternatives, improving the quality and quantity of natural resources and strengthening Natural Resource Management (NRM) and participation is a sure way to reducing conflict.

Thus alternative solutions to trans-humance agriculture of the Fulanis across West Africa, through the development of intensive small area grazing on hay and silage, and the establishment of standard ranches and grazing lands in the different parts of the country can ensure continuous feed for their animals. International organizations need to assist West African Governments in providing alternative solutions to the long and short range trans-humance agriculture of the cattle Fulanis. Obioha (2008) suggests that the grazing belt policies of West African Governments need to be religiously implemented if the conflict situation between the herdsmen and the settled arable farmers is to be minimized by mapping out areas of grazing which the arable farmers are prohibited to crop. This policy programme *inter-alia* provides a framework within which the herdsmen and the arable farmers can coexist with one another.

Stemming the south migration of the Sahara through intensification of aforestation projects as is being done in the Green Wall Sahara Project should be intensified, this is an important step towards stabilizing the Sudan and Sahel zone. By planting new trees could stabilize desert areas, wetlands and coastline vulnerable to desertification and floods, cut emissions, and even create jobs while boosting economic growth.

At this juncture, it is worthy of note that not all conflicts are destructive, some are actually beneficial. This is the view of Bartos and Wahr (2002), that too often, managing, reducing and resolving conflicts has simply deferred or postponed needed changes in power relations. It is therefore important for societies to build into itself a tolerance for 'healthy' conflict, like the United States whose members are growing increasingly tolerant of disagreements and differences and learning how to live with them more creatively and productively regardless of their differences. According to Coser (1956), both attraction and repulsion between groups are essential for social integration and continuity and healthy conflict is a part of societies' developmental process to stability.

Conflict remediation

Conflict remediation actions are those that address conflicts when they are already in place. This can be done by first identifying the root cause of conflict, which is important in designing the appropriate solution for the conflict. Acting on such causes will be a sure way of de-escalating the said conflict. Causes could be social, when individuals struggle for societal influence, ethnic domination, religious differences or political struggle. In this case creating an avenue for fair arbitration is important. Addressing equity and justice issues have always proved to be sure ways of addressing social conflicts. If they are due to economic concerns, the implementation of programmes and quick interventions which guarantees employment and livelihood options for the citizens should be explored. In developed economies the use of effective social security options are put in place to address economic conflicts. In this case individuals feel secures and cared for and have no need to worry for economic security. In the case the conflict arises due to the struggle for scarce resources, the establishment of good property right system. Delineating and policing resource use to avoid overexploitation and misuse is recommended. And where it is due to natural phenomenon like climate change or natural disaster, implementation of good adaptation mechanisms to cushion the effect of the menace is vital towards conflict de-escalation. In general, putting in place transparent and efficient governance systems cannot be over emphasized in the quest for conflict remediation. Others immediate

approach are the provision of immediate relief materials, food and good incentives to stop conflict and dispute resolution to take early action to defuse both imminent threats and broader instability are good options in reducing or stopping conflict. This should be followed by sincere and unbiased actions to resolve the existing conflict. Whatever the case may be the provision of prompt and adequate security to prevent or de-escalate conflict remain key to conflict management in any society, de-escalation should be followed by dialogue and mediation to build mutual trust. In this regard UNIFTPA, (2012) is of the opinion that direct conflict management can be by dialogue, systematic data collection and early warning systems, information sharing on the status of the disputed resource, joint assessment organized by impartial, independent third parties that are acceptable to both sides, joint management plans, legal binding agreements and practical dispute resolution support. The approach of integrative bargaining should be adopted, which permits each party to discover common and divergent interests, which are met collaboratively through joint brainstorming and creation of new options (Bartos and Wahr, 2002). It is important that one should be clear about what is required to be done by the factions and how it can be made more likely that the things will be done in order to bring about the desired future. And agreement reached should be made operational by putting adequate mechanism for its implementation.

Post conflict management

The primary aim of conflict resolution should be to sustain the peace and prevent future occurrence. This can be done by implementing enduring policies and programmes that are targeted at addressing the root causes of conflicts and providing supports to those affected by conflict, especially women, children and the disabled. When conflict cases are resolved, there is the tendency for a breakdown of law and order if the issues that caused the conflict in the first instance are not addressed. Stakeholders should carry out research on the said conflict by engaging the stakeholders in the conflict objectively using independent assessors who are not biased about the conflict in order to fashion out a strategy for lasting solution. Agreements should be reached through give-and-take negotiations and instituting binding realistic treaties and measures (incentives) to enforce them. There should be constant supervision and monitoring of the situation to ensure all is working according to plan.

Some of these strategies that can ensure long lasting peace in conflict situations are promoting and protecting rights of local people to natural resource, fostering their greater participation in decision making, promote policies which encourage equality and inclusion. Design programmes which make governments more responsive

and accountable to poor and excluded groups, improving access to justice for poor and excluded people, protecting the right of women and the less privileged in the society. The likelihood of violent conflict can also be reduced if we strengthen ways of managing disputes between individuals and groups fairly and speedily. This can be done by using local self-help organizations; castes, age grades, religious bodies, traditional rulers, local opinion leaders and chiefs, masquerade cults.

It is also possible to achieve conflict resolution by promoting mutual trust and cooperation in resource use, access and management. For example, in the case of Nigeria, Nyong (2007) advocate for the adoption of the Hadejia-Nguru Wetland Conservation Project strategy in resolving crop farmers – pastoralists' conflicts in the area. Realizing that a major source of this conflict was the lack of access to fodder for livestock the project promoted the cultivation of fodder by the farmers to sell to the pastoralists at a subsidized rate. Such a strategy should be modified and replicated in similar conflict situations.

Finally, the emerging strategies in the management of West Africa's violent conflicts have a strong foundation in African traditional cultures. Contrary to general belief in western paradigms, every African community has capacities for promoting mutual understanding and peaceful coexistence (Lauer, 2007). This is true of many pre-colonial African societies that used various indigenous knowledge and institutions to advance harmonious co-existence in the society, such social assets should be harnessed and modified o suit present day realities. Uncritical adoption of Western approaches to conflict management has adversely affected the stability and development of many African societies. Traditional strategies largely conform to the principles of compromise and collaboration and emphasis is placed on internalized values such as traditional oaths, rewards, vigilantes, informal settlements, checks and balances, decentralization, effective communication, good governance, honesty, openness, empathy, community solidarity, and individual loyalty to the group which promote greater feeling of belongedness and mutual trusts among groups. So, we need to go back to the basics and see how best we can integrate our traditional conflict management strategies in modern conflict management processes and programmes.

Conclusion

Resource scarcity and conflict have been part of human existence; the link between the two no doubt has been documented by the famous work of Malthus and contemporarily by those of neo-Malthusianism. The paper established that most cases of violent conflicts in West Africa are traceable to the struggle for the control of dwindling and degraded natural resource base of the region, which breeds poverty and scarcity in the already socially and economically fragile situations. Based on these linkages a conflict pathway model was developed with comparative advantages other existing conflict models for its robustness.

It is noted that most measures used in resolving crisis situations in West Africa treat the effects of resource scarcity without addressing the root issue of land modification, unsustainable farming and deforestation. The violence in the Middle-belt of Nigeria, for example, which has taken the lives of thousands of people, rendered many refugees and destroyed properties worth billions of Naira, does not have ethnic or political origin; the root is in resource scarcity and the struggle for possession. The paper suggests that socio-ecological crisis can be greatly reduced by improving and restoring degraded rural lands, provision of alternative means of livelihoods for the poor, education, employment, social security, making available basic infrastructure such as electricity, roads, piped water and processing of agricultural products, coupled with effective government policies and political processes. These conditions will help to reduce the feeling of insecurity and address the root causes of conflict in the West African sub region and other natural resource dependent systems.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

André C, Platteau J (1998). Land relations under unbearable stress: Rwanda caught in the Malthusian trap. J. Econ. Beh. Organ, 34(1):1–47.

Atta-Asamoah A, Aning EK (2011). Demography, environment and conflict in West Africa ECOWAS and the dynamics of conflict and peace building. In Thomas Jaye & Stella Amadi (Eds.) *Consortium for Development* Partnership (CDD), Dakar, CODESRIA, P. 252.

Baechler G (1998). Why environmental transformation causes violence: A synthesis. Environmental change and security project report, (Spring) 4:24-44.

Baechler G (1999). Violence through environmental discrimination. Dordrecht, Kluwer Academic Publisher.

Bächler G, Böge V, Klötzli S, Libiszewski S, Spillmann KR (1996). Environmental degradation as a cause of war: Ecological conflicts in the third world and peaceful ways of resolving them. Zurich: Rüegger.

Ban KM (2007). A climate culprit in Darfur. The Washington Post, 16 June, P. A15.

Barnett J, Adger N (2007). Climate change, human security and violent conflict. Pol. Geog. 26(6):639-655.

Bathos OJ, Wehr P (2002). *Using Conflict Theory*. Cambridge University Press.

Bernauer T, Koubi1 V, Böhmelt T (2011). Migration and global environmental change. SR12: Environmental changes and violent conflict, UK Government Foresight Project, Government office for science.

Blench R (2004). Natural resource conflict in North-Central Nigeria: A handbook and case studies, United Kingdom, Cambridge: Mallam Dendo Ltd.

Buhaug H, Gleditsch NP, Theisen OM (2008). Implications of climate change for armed conflict. World Bank Workshop on Social Dimensions of Climate Change. Washington, DC: World Bank.

Brooks N (2006). Climate change, drought and pastoralism in the Sahel– a discussion note for the World Initiative on Sustainable Pastoralism,"WISP.

Brown LR (2006). The earth is shrinking: Advancing deserts and rising seas squeezing civilization. Earth Policy Institute. Accessed on 05-05-11 from http://www.earth-policy.org/Updates/2006/Update61.htm.

Brown O, Crawford A (2008). Assessing the security implications of climate change for West Africa Country case studies of Ghana and Burkina Faso. International Institute for Sustainable Development.

Collins R (1974). The Basics of Conflict Theory 2. Conflict Sociology. New York: Academic Press, 1974, pp. 56-61.

Coser LA (1956). The functions of social conflict Glencoe, IL: Free Press

Department for International Development (2005). Reducing poverty by tackling social exclusion: DFID.

Department for International Development (2006). Preventing Violent Conflict. DFID. London, UK.

De Soysa I (2000). Natural Resources and Civil War: Shrinking Pie or Honey Pot? Oslo, PRIO. Mimeo.

.de Soysa I, Gleditsch N, Gibson M, Sollenberg N (1999). To cultivate peace: agriculture in a world of conflict. Environmental Change and Security Project Report, 5:15e25.

Diamond J (2005). Collapse: How societies choose to fail or survive, New York: Viking.

Dollard J, Leonard WD, Neal EM, Mowrer OH, Rpobert RS (1939). Frustration and aggression, New Haven CT, Yale University Press.

Ehrlich AH, Gleick P, Conca K (2000). Resources and environmental degradation as sources of conflict. Draft background paper for working group 5: 50th Pugwash Conference on Science and World Affairs: Queens' College, Cambridge, UK, 3-8 August 2000.

European Climate Forum (ECF), Potsdam Institute (2004). *Report on the Beijing Symposium on Article 2*, September, 2004.

Fasona MJ, Omojola AS (2005). Climate Change, Human Security and Communal Clashes in Nigeria. Paper presented at Human Security and Climate Change An Intl Workshop Oslo, 21–23 June, 2005.

Filho WL (ed.) (2011). The Economic, Social and Political Elements of Climate Change,Climate Change Management, DOI 10.1007/978-3-642-14776-0_2, # Springer-Verlag Berlin Heidelberg.

Gendron R, Hoffman E (2009). Resource scarcity and the prevention of violent conflicts peace and conf. Rev. 4(1):1-13.

Gleick PH (1989). Climate change and international politicing developing countries',Ambio 18(6):333-339.

Gleick PH (1993). Water in the 21st century' in Peter HG (ed), Crisis: A Quick Guide to the World's Fresh Water Resources. Oxford & New York: Oxford University Press.

Gonzalez P (2001). Desertification and a shift of forest species in the West African Sahel. Clim. Res. 17:217-228.

Gough M (2002). Human security: the individual in the security question e the case of Bosnia. Contemporary Security Policy, 23:145-191.

Hardin G (1968). The tragedy of the commons. *Science* 162:243-1248.

Hauge W, Ellingsen T (1998). Beyond environmental scarcity: Causal pathways to conflict, J. Peace Res. 35(3):299-317.

Hendrix CS, Sarah MG (2005). Trends and triggers: climate change and civil conflict in Sub Saharan Africa, Paper prepared for the Conference on Human Security and Climate Change, Asker, Near Oslo, Norway, 21–23 June (downloaded from cicero.uio.no/humsec/papers/Hendrix.Glaser.pdf

Hilyard N (1999). Blood, babies and the social root of conflict, in Suliman M (ed) *Ecology, Politics* and Violent Conflict London Zeb Books Ltd.

Homer-Dixon TF (1991). On the threshold, environmental change as the cause of acute conflict. , Int. Sec. 16(2):76-116.

Homer-Dixon TF (1994). Environmental scarcity and violent conflict., evidence from cases, Int. Sec. 19(1):5-40.

Homer-Dixon T (1999). Environment, Scarcity, Violence. Princeton, NJ: Princeton University Press.

Homer-Dixon T, Blitt J (1998). Ecoviolence: Links among Environment, Population and *Security*. Lanham, MD: Rowman and Littlefield.

Human Security Report Project (2007). Human Security Brief 2007. Vancouver: Human Security Report Project, http://www.humansecuritybrief.info/HSRP_Brief_2007.pdf

Idinoba M, Nkem J, Kalame FB, Obeng ET, Gyampoh B (2010).

Dealing with reducing trends in forest ecosystem services through a vulnerability assessment and planned adaptation actions. Afr. J. Environ. Sci. Technol. 4(7):419-429.

Integrated Regional Information Network (IRIN) (2004). Africa: Diminishing water resources could fuel conflicts – Experts, Integrated Regional Information Network, 5 November. http://www.globalpolicy.org/security/natres/water/2004/1105diminishing.htm.

Kahl C (1998). Population growth, environmental degradation, and state-sponsored violence: The case of Kenya, 1991–93', Int. Sec. 23(2):80-119.

Kahl C (2006). States, Scarcity, and Civil Strife in the Developing World. Princeton: Princeton University Press.

Kameri-Mbote P (2004). Environment and conflict linkages: An overview. Geneva. International Environmental Law Research Centre. http://www.ielrc.org/activities/presentation_0410.htm

Koubi V, Bernauer T, Kalbhenn A. Spilker G (2012). Climate change, economic growth and conflict. J. Peace Res. 49(1):113-127

Krummenacher H (2008) Environmental factors as triggers for violent conflict: empirical evidence from the 'FAST'Data Base. Bern: Swisspeace.

Lauer H (2007). Depreciating African political culture. J. Black Stud. 38(2):288–307.

Lepird E, Canny S, Saldana M (2012). Conflict Theory. www.slideserve.com/Pat_Xavi/conflict-theory

Levy MA, Thorkelson C, Vörösmarty C, Douglas E, Humphreys M (2005). Freshwater availability anomalies and outbreak of internal war: results from a global spatial time series analysis', Paper prepared for the Conference on Human Security Near Oslo, Norway, 21–23 June. http://www.cicero.uio.no/humsec/papers/Levy_et_al.pdf,

López A (1999). Environmental Change, Security, and Social Conflicts in the Brazilian Amazon, Environmental Change Security Project Report 5:26–33.

Mack RM, Snyder RC (1973). The analysis of social conflict--toward an overview and synthesis. In Conflict resolution through communication, F. E. Jandt. New York: Harper and Row.

Martin A (2005). Environmental conflict between refugee and host communities. J. Peace Res. 42:329-346.

Matthew R (2008). Resource scarcity: Responding to the security challenge. New York: International Peace Institute. http://www.ipacademy.org/asset/file/304/rscar0408.pdf

Miguel E, Satyanath E, Sergenti E, (2004). 'Economic shocks and civil conflict: An instrumental variable approach', J. Polit. Econ. 112(4):725-753.

Mongabay (2006). Niger: Tropical Rainforest Country Profile. Accessed on 16/10/2011at http://rainforests.mongabay.com/20niger.htm.

Moorhead R (1989). Changes taking place in common property resource management in the inland Niger Delta of Mali, in Berkes, F., (ed) *Common Property Resource*, London, Belhaven press, pp. 256-272

Myers N (1993). Ultimate security: The Environmental Basis of Political Stability. New York: Norton.

Niasse M (2007). Climate-induced conflict risks over shared waters in West Africa. In: The third international conference on climate and water, Helsinki, Finland, 3–6 Sept 2007.

Nyong A (2007). Climate-Related Conflicts in West Africa, in Report from Africa. Population, Health, Environment and Conflict Report 12:2006–2007.

Obioha E (2008). Climate change, population drift and violent conflict over land resources in north eastern Nigeria. J. Hum. Econ. 23(4):311-324.

Ohlsson L (1999). Environment scarcity and conflict: a study of Malthusian concerns. Department of Peace and Development Research, Goteborg University.

Ohlsson L (2003). The risk of livelihood conflicts and the nature of policy measures required, in Nicholas N. K. et al., eds , Seeds of True Peace: Responding to the Discontents of a Global Community Washington, DC: Carolina Academic Press.

Okpi A (2010). Climate change threat to Nigerian Cooperate existence Accessed on 30-05-11 from http://234next.com/csp/cms/sites/Next/News/5641414-147/climate_change_as_threat_to_nigerias.csp

Olori T (2002). Desertification Threatens Economy, Food Security Accessed on 30-05-11 from http://ipsnews.net/riomas10/2608_8.shtml.

Olufemi FJ, Samson AO (2012). Climate change, environment and conflicts in Nigeria. *Brit*. J. Arts. Soc. Sci. 6(1):2046-9578

Onuoha FC (2008a). Environmental degradation, livelihood and conflict: a focus on the implications of the diminishing water resources of Lake Chad for North-Eastern Nigeria. Afr. J. Confl. Res. 8(2):35-62.

Onuoha FC (2008b). Saving Africa's shrinking lakes through inter-basin water transfer: reflections on the Lake Chad replenishment project. Nig. J. Int. Aff. 34(2):65–84.

Onuoha FC (2010). Climate change, population surge and resource overuse in the Lake Chad area Implications for human security in the north-east zone of Nigeria. Institute for Security Studies (ISS) report, P. 10.

Percival V, Homer-Dixon T (1998). Environmental scarcity and violent conflict: the case of South Africa. J. Peace Res. 35:279-298.

Petzold-Bradley E, Carius A, March A, Vincze A (2001). Theoretical linkages and policy approaches to the environment and security debate: providing an overview. in Petzold-Bradley E, Carius A, Vincze A (eds), Responding to Environmental Conflicts: Implications for Theory and *Practice*. Dordrecht: Kluwer pp.1-19).

Raleigh C, Urdal H (2005). Climate change, environmental degradation and armed conflict. Paper prepared for the Conference on Human Security and Climate Change, Asker, Near Oslo, Norway, 21–23 June (downloaded from http://www.cicero.uio.no/humsec/papers/Raleigh&Urdal.pdf, last accessed 6 October 2005).

Renner M (1997). Fighting for Survival: Environmental Decline, Social Conflict, and the New Age of Insecurity. New York: Norton.

Salehyan I (2008). From climate change to conflict? No consensus yet. J. Peace Res. 45:315-326.

Sayne A (2011). Climate change adaptation and conflict in Nigeria. United States Institute of Peace, Apecial report, P. 274.

Scoons I (1995). Living with Uncertainty: New Dimensions in Pastoral Development in Africa. London, Intermediate Technology Publisher

Shetima AG, Tar UA (2008) Farmer-Pastoralist conflict in West Africa: Exploring the causes and consequences. Info. Soc. Just. 1(2):163-184

Spillman KR (1995) From environmental change to environmental conflict. In: K. Spillmann and G. Bächler (eds), *Environmental Crisis: Regional Conflicts and Ways to Cooperate*. Zurich: Centre for Security Studies.

Stern N (2006). The Economics of Climate Change: The Stern Review. Cambridge, MA: Cambridge University Press.

Suhrke A (1993). Pressure Points: Environmental Degradation, Migration and Conflict. Unpublished manuscript, Chr. Michelsen Institute.

Suliman M (1993). Civil war in the Sudan: From ethnic to ecological conflict', The Ecol. 23(3):104-109.

Tamas P (2003). Water resource scarcity and conflict: review of applicable indicators and systems of reference UNESCO Technical Documentary in Hydrology, PC-CP series P. 21 (SC-2003/WS/49)

Theisen OM (2006). Renewable resource scarcity and internal armed conflict: A quantitative reassessment. Master's thesis in political science, Spring 2006. ISS, NTNU & CSCW/PRIO

Transboundary Freshwater Dispute Database (2000). Facing the facts: assessing the vulnerability of Africa's water resources to environmental change. UNESCO/UNEP Publications, Geneva

UNCCD (2004). Ten years on: UN marks World Day to Combat Desertification; Observances worldwide on June 17, 2004 http://www.unccd.int/publicinfo/pressrel/showpressrel.php?pr=press01_06_04

United Nations Environment Programme (UNEP) (2006). Africa's lakes: atlas of our changing environment, Nairobi: UNEP, 2006.

UNEP (2008). AFRICA Atlas of our changing environment. UNEP, Nairobi. Accessed on

15/06/2008 at http://na.unep.net/AfricaAtlas/AfricaAtlas/

UNEP (2009). From conflict to peace building: the role of natural resources and the environment. Nairobi: UNEP.

UNEP International Organization for Migration, Office for the Coordination of Humanitarian Affairs, United Nations University and the Permanent Interstate Committee for Drought Control in the Sahel (2011). Changes in climate trends impacting livelihoods and food security in the Sahel and West Africa: Joint study calls for major investments in climate change adaptation to reduce the risk of conflict and forced migration, Durban conference, 5 December. 2011 .http://www.unep.org/Documents.Multilingual/Default.Print.asp?DocumentID=2661&ArticleID=8971&l=en

UN Interagency Framework Team for Preventive Action (2012). Renewable resources and conflict. UNEP

United Nations Economic Commission for Africa (2008). Africa Review Report on Drought and Desertification. UNECA , Addis Ababa, Ethiopia. Accessed on 30-05-11 from http://www.uneca.org/eca_resources/publications/books/drought/index.htm.

United States State Department (2009). Nigeria: Human rights report. Washington, DC: U.S. State Department, 2009

Urama KC, Ozor N (2010). Impacts of climate change on water resources in africa: the role of adaptation. Accessed on 25th January, 2012 from http://www.ourplanet.com/climate-adaptation/Urama_Ozorv.pdf.

Wellesteen P (2002). Understanding Conflict Resolution: War, Peace and Global System, Sage Publications, London

Yusuf S (2009). Desertification calamity in Northern Nigeria: Threat to Nigerian economy Nigerian Compass 13 October, 2009.

African Journal of Agricultural Research

Related Journals Published by Academic Journals

- *African Journal of Environmental Science & Technology*
- *Biotechnology & Molecular Biology Reviews*
- *African Journal of Biochemistry Research*
- *African Journal of Microbiology Research*
- *African Journal of Pure & Applied Chemistry*
- *African Journal of Food Science*
- *African Journal of Biotechnology*
- *African Journal of Pharmacy & Pharmacology*
- *African Journal of Plant Science*
- *Journal of Medicinal Plant Research*
- *International Journal of Physical Sciences*
- *Scientific Research and Essays*