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Evaluation of Pepper Intercropped with Tall-Companion Crop in the Management of Pepper Veinal Mottle Virus Potyvirus Disease and its Vectors on Cultivated Pepper (*Capsicum annuum* L.) in Nigeria

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Abstract: Three intercropping models (cassava/pepper, plantain/pepper and maize/pepper) were evaluated for the management of pepper veinal mottle potyvirus disease on cultivated pepper in Nigeria between 2004-2006. A viral susceptible variety accession NHV1-E96 obtained from National Horticultural Research Institute, Idi-Ishin, Ibadan was intercropped with plantain, cassava and maize, respectively. A 12 month maturing local variety of cassava called The Groom 90 Days Maturing maize variety (DMSR-1) and French long horn plantain variety (PITA 14) were used for this experiment. Data were taken on viral disease incidence and severity and the pepper fruit yield in each treatment plot which were later subjected to statistical analysis. There was no significant difference in the three year data result obtained on the field. Therefore, averages for the 3 years data where subjected to statistical analysis. The viral disease incidence and severity showed a significant difference at a probability of <5% in all the treatment used. Viral disease incidence and severity were relatively higher in the sole pepper crop compared with pepper intercropped with plantain, cassava or maize. The sole pepper cropping recorded 42% age disease incidence and 23.3% severity compared with maize/pepper intercrop that recorded 10% disease incidence and 8% severity while cassava/pepper and plantain/pepper intercropping models had 5 and 8.33% disease incidence and 4 and 7% severity, respectively. There was also a high significant difference in the yield of pepper in the treatments ($p<0.05$). Sole pepper cropping recorded a yield of 1.54 tons ha^{-1} while maize pepper intercrop recorded the highest pepper fruit yield of 5.98 tons ha^{-1}. The success of the PVMV disease management evaluated in this study was judged by the extent of reduction in number of diseased plants and by an increase in vigor of the cultivated pepper crop, coupled with increase in fruit yield and quality. This signifies that for devising effective viral disease management for any crop it is important that the vectors of the virus present in that particular agro-ecological zone are effectively shielded away from the target crop.

Key words: Evaluation, incidence, management, pepper veinal mottle virus, severity, Nigeria

INTRODUCTION

Farmers have practiced cultural and mechanical pest control based on trial and error as part of their land management systems (Fajinmi and Odebode, 2007). In developing countries intercropping is extremely widespread owing to its advantages. Most farmers practice the intercropping farming system because of the higher gross return per unit area of land under use than in sole cropping.

Many factors have to be taken into consideration before applying intercropping model as an integrated approach in viral disease control such as PVMV. Successful crop mixtures in the intercrop share available resources over time and space in a way that they exploit variation between component crops in such characteristics as rates of canopy development, width and height, photosynthetic adaptation of canopies to irradiation and rooting depth (Fajinmi, 2006).

According to Ikeorgu (1984) and Adeyemi (1991), intercropping does not always result in increased yield of each component crop in a mixture, rather a reduction in yield of one component crop and an increase in yield of others. This could be avoided as suggested by Fajinmi (2006) that optimal natural resource use could only be attained when mixtures are not consisting of highly

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competitive crops. Intercropping stability over space and time is likely to be favoured by the choice of less aggressive cultivars of similar final canopy heights. Therefore, Osiru and Ezumah (1991) revealed that temporal and special compatibility is particularly important and can be enhanced by differences in growth cycles of the companion crop.

In traditional cropping systems, vegetables such as *Amaranthus cruentus, Celosia argentia, Corchorus olitorus, Solanum* sp. *Lycopersicum esculenta, Abelmoschus* sp. and *Capsicum* sp. are commonly intercropped with food crops like *Dioscorea* sp. *Zea mays, Manihot* sp. and *Musa* sp. to enhance increase in economic gains (IART, 1991).

Intercropping has an influence on the population build up of insect pests. It reduces the incidence of pest in cases where the crops used in intercropping are not hosting the same insect species. In contrast, intercropping of crops that host the same insect pests increase the incidence of these pests. The reduction of pest incidence with intercropping of non-host plants may partly be explained by the increased diversity of the agro-ecosystem combined with changes in the microclimate of the field (Fajinmi, 2006). Other factors such as different planting times for the crops are also important. The knowledge of all these factors will significantly contribute to an effective integrated approach in the control of PVMV diseases of pepper.

Therefore, this study was aimed at evaluating the efficacy of intercropping pepper with a tall companion crop such as maize, cassava and plantain as a cultural management technique in reducing the effect of the PVMV disease on the yield and other agronomic features of cultivated pepper.

MATERIALS AND METHODS

A viral susceptible variety accession NHV1-E96 obtained from National Horticultural Research Institute (NIHORT), Idi-Ishin was intercropped with plantain, cassava and maize, respectively between 2004-2006 on NIHORT experimental research field. A local variety of cassava called The Groom 90 Days Maturing maize variety (DMSR-1) and French long horn plantain variety (PITA 14) were used for this experiment.

The pepper seedlings were raised in a screened greenhouse and transplanted within the intercropping companion plants and sole pepper plots at 5 weeks old. The pepper seedlings were transplanted after 2 weeks of planting maize after 6 weeks of planting cassava cuttings and after 8 weeks of planting plantain suckers. The cassava variety used was an erect non-branching local variety which takes between 10-12 months to maturation and harvesting. The maize variety was planted at two seeds per hole which was later thinned down to one after seven days. Also the leaves of the cassava varieties used were trimmed at 8 weeks interval.

Four treatments were used in this experiment: Sole pepper, pepper/cassava intercrop pepper/plantain intercrop and pepper/maize intercrop. Plot size used was 10×10 m and inter-plot spacing was 4 m. In the cassava/pepper intercropped plot, the cassava cuttings were planted at a spacing of 1.5×1.5 m (49 cassava stands) per plot and the pepper was intercropped within the cassava variety at spacing of 50×60 cm, 300 pepper stands per plot (population rate of 40,000 pepper plants ha^{-1} and 10,000 cassava plants ha^{-1}, respectively).

The plot was replicated 3 times and randomized. In plantain/pepper intercrop plot, similar population density was used for the pepper intercropped and same planting distance while plantain was planted at a spacing of 3×3 m, 12 plants per plot (1,111 plantain plants ha^{-1}). The plot was replicated 3 times and randomized. In maize/pepper intercrop, same planting distance used for cassava that is 1.5×1.5 m was used for maize and also same planting distance used for pepper was used 50×60 cm. The plot was replicated three times and randomized.

Pepper was planted in the sole pepper plot at a spacing of 50×60 cm, 300 pepper stand per plot (at a population of 40,000 pepper plants ha^{-1}). The plot was also replicated three times and randomized. The experiment was a randomized complete block design. There was no insecticide application and no fertilizer application. Weeding of the plot was done at 3 weeks interval after transplanting.

To increase inoculum's density, two rows of 4 weeks old pepper seedlings of bell fruit shaped Tattasai cultivars each mechanically inoculated with PVMV and confirmed through serological test using PAS-ELISA were planted at a spacing of 50×60 cm 2 m away on both sides of the main treatment plots.

PVMV disease incidence and severity were then monitored on the pepper plants leaving the guard rows in each treatment plot by using a modified formula-grading scheme from Steel and Torrie (1980) and Nelson *et al.* (1999) for disease incidence and severity:

- No disease symptoms
- Leaf mottling
- Chlorosis/leaf mottling
- Stunting/severe mottling/leaf bunching
- Leaf defoliation

$$\text{Disease severity} = \frac{1\times P_1+2\times P_2+3\times P_3+4\times P_4+5\times P_5}{N\ (G\text{-}1)}\times\frac{100}{1}$$

$$\text{Percentage disease incidence} = \frac{N\text{-}n}{N}\times\frac{100}{1}$$

Where:

P_1-P_5	=	Total number of observed plants in each disease symptoms grading per plot
G	=	Number of grading = 5
N	=	Total number of observations
n	=	Total number of plants with no disease symptoms

Pepper leaf samples showing virus disease symptoms were sampled from pepper plants been monitored for serological analysis to confirm the presence of PVMV. The yield of the pepper fruit was also taken in each plot (i.e., in the intercropped and the sole pepper plot) for a period of 12 weeks. Other agronomic data taken included; plant height, leaf number, internode's distance and fruit number. The plots were rain-fed and the experiment was repeated for 3 years. The averages for the 3 years data were recorded and the results were analyzed statistically. Using analysis of variance procedure, the means were separated by using Duncan multiple range tests at 5% significance level. The land equivalent ratio and the yield proportion of pepper in each of the intercrop were calculated as described by Mead and Willey (1980).

Virus detection: Protein-a sandwich ELISA (PAS-ELISA) (KPL Technical Guide for ELISA protocols on line 1999/2000 edition and IITA Virology laboratory modified protocols) was used for the detection of the presence of PVMV on pepper leaf samples collected from pepper plants. The PVMV antibody used was AAB 328 antiserum diluted in ratio 1:1000 with Phosphate Buffered Saline (PBS-T) (0.05% Tween 20: pH 7.4: 8.0 g NaCl, 0.2 g KH_2PO_4, 1.1 g Na_2HPO_4 0.2 g KCl, 0.2 g NaN_3 in 1 L H_2O+ 0.5 mL Tween 20 (0.05%)) collected from the Virology Laboratory of the International Institute of Tropical Agriculture (IITA) Ibadan.

Virus indexing protocols: One hundred micro liter of protein A at 1 μg mL^{-1} in coating buffer was dispensed into each well of ELISA plate. The plate was then incubated at 37°C for 2 h. The plate was washed three times with PBS-T after the incubation period. About 100 μL of PVMV polyclonal (AAB 328) antiserum diluted 1:1000 in PBS-T was added to each of the ELISA plate and then incubated at 37°C for 2 h. After incubation the ELISA plate was washed three times with PBS-T.

One hundred micro liter of antigen (e.g., sap) ground in PBS-T +2% PVP (Polyvynil pyrolidone) was added into each of the wells of the ELISA plate and incubated overnight at 4°C. The plate was washed three times with PBS-T and 100 μL of PVMV polyclonal (AAB 328) antiserum diluted 1:1000 in PBS-T was added into each of the wells. The plate was further incubated at 37°C for 2 h after which it was washed three times with PBS-T. About 100 μL of protein, A-alkaline phosphatase conjugate diluted 1:1000 in conjugate buffer ($^1/_2$ PBS +0.05% Tween 20+ 0.02% egg albumin +0.2% PVP +0.02 g NaN_3) was added per well and the plate incubated at 37°C for 2 h. The plate was washed three times with PBS-T. About 200 μL of 0.5-1 mg mL^{-1} of p-nitrophenyl phosphate substrate in substrate buffer (97 mL diethanolamine +800 mL H_2O +0.2 g NaN_3 add HCl to give pH 9.8) was added per well and incubated at room temperature for 30 min 1 h.

For all incubations plates were covered with ELISA cover plates to avoid edge effects and to maintain uniform temperature. Healthy pepper plants (*Capsicum* sp.) were used as negative control while PVMV infected *Capsicum* sp. were used as positive control.

After 1 h, the absorbance was measured at 405 nm using multiscan ELISA reader. The samples were considered positive when the ELISA reading exceeded that of the healthy control by or was at least twice the reading for the healthy control.

RESULTS AND DISCUSSION

There was no significant difference in the 3 years data result obtained on the field. Therefore, averages for the 3 years data where subjected to statistical analysis. The intercropping models (maize/pepper, cassava/pepper and plantain/pepper) showed a significant effect in the reduction of viral disease incidence and severity in the pepper intercropped compared with that of sole pepper cropping (Table 1).

Viral disease incidence and severity were relatively higher in the sole pepper crop compared with pepper intercropped with plantain, cassava or maize. The sole pepper cropping recorded 42% disease incidence and disease incidence and 8, 4 and 7% disease severity in the

Table 1: Percentage diseases incidence and severity of PVMV on pepper intercropped with maize, cassava and plantain crops

Intercropped pepper	Viral disease severity (%)	Viral disease incidence (%)
Sole pepper	23.30^a	42.00^a
Maize/pepper	8.00^b	10.00^b
Cassava/pepper	4.00^c	5.00^c
Plantain/pepper	7.00^b	8.30^{b,c}
SE±	2.86	4.54

S.E± = Standard Error, means with the same letter are not significantly different from each other at 5% level of probability

Table 2: Agronomic growth parameter characteristics of pepper intercropped with maize, cassava and plantain crops

Intercropped pepper	Number of days to flowering	Average number of fruit/ plant	Fruit length (cm)	Fruit girth (cm)	Fruit wall thickness (cm)	Average number of seeds/ fruit	Average fruit weight (g)	Average plant height (m)	Fruit yield tons ha^{-1}	Land equivalent ratio tons ha^{-1}	Proportion of pepper yield in the ntercrop
Sole pepper	56.00^{b}	90.00^{b}	10-12^{c}	2.0-3.5^{b}	0.2-3.5^{a}	40.0^{a}	3.00^{c}	0.60^{c}	1.54^{c}	-	-
Maize/pepper	70.00^{a}	120.00^{a}	14-15^{a}	2.5-4.0^{a}	0.2-0.35^{a}	42.0^{a}	5.50^{a}	1.40^{a}	5.98^{a}	1.94	0.21
Cassava/pepper	73.00^{a}	65.00^{c}	12-13^{b}	1.5-2.0^{c}	0.2-0.25^{a}	40.0^{a}	3.50^{b}	1.00^{b}	3.75^{b}	1.22	0.13
Plantain/pepper	74.00^{a}	70.00^{c}	12-13^{b}	1.5-2.0^{c}	0.25-0.35^{a}	42.0^{a}	3.90^{b}	1.12^{b}	4.63^{b}	1.50	0.16
SE±	0.34	4.25	0.64	0.34	0.16	2.4	0.22	0.50	0.43	-	-

Means with the same letter are not significantly different from each other at 5% level of probability

Table 3: Correlation similarity coefficient matrix of percentage disease incidence, severity and yield of pepper intercropped with maize, cassava and plantain

	Disease severity(%)	Yield	Disease incidence(%)
Disease severity(%)	1.000	-	-
Yield	-0.539	1.000	-
Disease incidence (%)	0.753**	-0.706*	1.000

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed)

maize/pepper, cassava/pepper and plantain/pepper, respectively (Table 1). There was a significant difference in viral disease incidence and severity at a probability <5% in all the treatments.

Sole pepper cropping recorded a yield of 1.54 tons ha^{-1} while maize pepper intercrop recorded the highest pepper yield of 5.98 tons ha^{-1} (Table 2). There was a high significant difference in the yield of pepper in the treatments ($p<0.05$). The yield of pepper in the maize/pepper intercrop had the highest land equivalent ratio of 1.94 tons per ha^{-1} compared with the yield of pepper in the plantain/pepper intercrop that had 1.5 tons ha^{-1} and cassava/pepper intercrop having 1.22 tons per ha^{-1} (Table 2).

The total land equivalent ratio was recorded to be 4.65 tons ha^{-1}. The yield proportion of pepper in maize/pepper intercrop was 0.21 tons ha^{-1} and that of cassava/pepper was 0.13 tons ha^{-1}, while that of plantain/pepper was 0.16 tons ha^{-1} (Table 2).

There was a significant negative correlation at probability <0.05 between disease incidence, severity and the fruit yield of pepper (Table 3). This showed that there was an interaction between the disease incidence and severity and the yield of pepper because increasing disease incidence and severity resulted in significant ($p<0.05$) reduction in the fruit yield of pepper (Fig. 1 and 2).

Intercropping pepper with a tall companion crop drastically reduced the viral disease incidence and severity in pepper intercropped within. The maize plant served as a barrier for the pepper plant from the invading aphids. Winged females alight fairly indiscriminately on hosts as expected for a polyphagous species, although they have a landing preference on yellow and yellow-

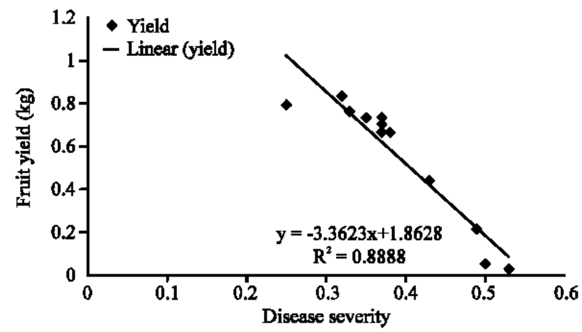

Fig 1: Linear curve estimation showing reduction in potted pepper fruit yield as disease severity increased

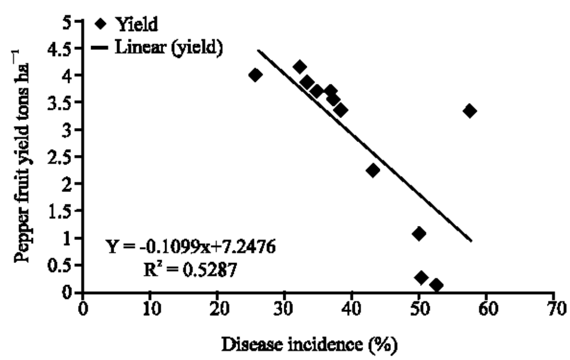

Fig 2: Linear curve estimation showing reduction in the fruit yield of field cultivated pepper as disease incidence increased

green surfaces (Kaakeh and Hogmire, 1991; Halima-Kamel and Hamouda, 1993). Taiwan Agricultural Research Institute (1983) also confirmed the use of intercropping pepper with corn in alternate rows to significantly reduce virus incidence compared with other treatment such as the use of reflective mulches, mineral oils and insecticides sprays. Also intercropping with Banker plants comprising wheat or barley or a tall companion crop (like maize) enhanced the establishment of *Aphid colemani* and *Aphid apidimyza* which predates on *Aphid gossipii* (Bennison, 1992; Bennison and Corless, 1993; Mansour *et al.*, 2000). Also in a survey carried out in some states in the northern part of Nigeria, it was observed that viral disease incidence was drastically reduced in pepper/sorghum and pepper/maize crop mixture compared with sole pepper cropped which had high significant viral disease incidence (Alegbejo and Uvah, 1987).

Maize intercropped with pepper showed effective management control in reducing viral disease incidence in pepper with an increase in yield compared to other treatments used. The reduction of virus-transmitting aphids is additional advantage in intercropping pepper with tall compatible crop (Bennison and Corless, 1993; Midmore, 1993; Mansour *et al*., 2000). The high disease incidence and severity in the sole pepper crop led to high significant reduction in crop yield compared with pepper yield in the intercrop. A similar observation was made by Fuchs and Minzenmayer (1995) that there could be >25% reduction in fruit yield in crops with high disease incidence and severity.

It could be misleading to argue that maize/pepper intercrop is better than cassava/pepper intercrop or plantain/pepper intercrop on the basis of higher land equivalent ratio or reduction in disease incidence level because plantain/pepper intercrop might be preferred if the yield proportion of pepper desired by the farmer is 0.16 tons ha^{-1}. Thus, it may be relatively easy to interpret a single land equivalent ratio value but there are difficulties in comparing different ones. Also cassava/pepper intercrop had the least viral disease incidence level 4 and severity 5% compared to other pepper intercrops, therefore it is only the farmer that can now decide on its choice of companion plant. Though, maize pepper intercrop had the highest pepper yield proportion of 0.21 tons ha^{-1}, the choice of intercropping companion plant depends on the quality preference by the farmer.

CONCLUSION

The management control of PVMV disease is the prevention of crop losses as observed by this study to include control of vectors through intercropping with compatible barrier crops. A more sustainable approach in controlling aphid vectors as suggested by this study was the prevention of aphids reaching the crops. This was achieved by planting compatible barrier crops.

RECOMMENDATIONS

Vegetables many of which are eaten raw should be treated with a lot of caution; there should be maximum reduction in the use of pesticides. Vegetables are an important commodity, they are an integral part of almost all the many multiple cropping systems and most of these vegetables especially pepper are grown in the open field which exposes them to viral infection. Intercropping as an IPM component should be encouraged because of its favourable impact on maintaining general biological diversity in the face of the unreliability of varietals resistance. The concept of systems viral disease management needs to be progressively incorporated into the cropping systems used in order to reduce the need for all types of pest controls. Crops will need to be more carefully selected for their appropriateness to a specific regional ecology and the optimal cultivation patterns determined. The conservation of natural enemies should be recognized as one of the most important aspects of a sound agricultural management policy (Cenpukdee and Fukai, 1992).

Through intercropping compatible crops the yield per hectare of pepper and other vegetables could be increased drastically. Consumers are increasingly concerned about environmental issues such as pesticide use. Fortunately by using best management practices, the need for pesticides may be reduced and control of most plant diseases can be accomplished without pesticides. It should complement other production practices and finally if the control measure opposes other good farming practices, a compromise may be necessary as suggested by Jerger (2004).

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