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THE USE OF PLANT LATEX AS A BIOCONTROL AGENT FOR THE INHIBITION OF *Moroccan watermelon mosaic virus* (MWMV) SOURCED FROM THREE LATEX PRODUCING PLANTS.

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ABSTRACT

An experiment on the efficacy on lattices from three commonly found plants in Calabar, Cross River State, Nigeria- *Jatropha curcas* L. (Euphorbiaceae), *Carica papaya* L. (Caricaceae) and *Thevetia neriifolia* L. (Apocynaceae) was carried out to test their ability to inhibit the *Moroccan watermelon mosaic virus* (MWMV) and on the transmission efficiency of the virus by *Aphis spiraecola*. MWMV is a potyvirus known to infect Curcubits in the South-south and South-east regions of southern Nigeria. Preliminary test on the plant lattices was carried out to investigate their ability to inhibit the virus and results showed that the percentage inhibition from *C. papaya* was highest with 94.26%, followed by 71.5% for *J. curcas* and 15.6% for *T. neriifolia*. The inhibition percentages for *J. curcas* and *C. papaya* were significant ($P \leq 0.05$) however that of *T. neriifolia* was not ($P \geq 0.05$) hence its use was discontinued. Also, the Minimum Inhibitory Concentration (MIC) for the lattices of *J. curcas* and *C. papaya* being the two plants with significant inhibitions was determined. Results showed that as the concentration of the lattices was increased from 0.25 mg/ml to 1.0 mg/ml, inhibition from *C. papaya* latex increased from 73.7% to complete inhibition (100%) and from 61.45% to 81.02% for *J. curcas* latex. Furthemore, the effect of latex treatment on the efficiency of the transmission of MWMV by *Aphis spiraecola* was also investigated. The results showed a common pattern of marked reduction in transmission efficiency of the virus by the aphid with the latex from *C. papaya* showing more inhibition (as high as 90%) than the latex from *J. curcas* (as high as 75%). The infection of plants by MWMV affect yield through a reduction in photosynthetic efficiency therefore having found the lattices of *C. papaya* and *J. curcas* capable of inhibiting the virus they can by extension reduce infection.

Keywords: Latex, Inhibition, MWMV, Calabar, *Aphis spiraecola*, *C. papaya*, *J. curcas*, *T. neriifolia*
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1. INTRODUCTION

According to The International Plant Protection Convention (IPPC), in 2012 global sales of chemical pesticides for plant pest control was about $45 billion per annum. Walia and Dikshit, (2009) reports of India ranking 10th in the world terms of pesticide use. Their findings reveals that when used, only 0.1% of the pesticide reaches the target pest with over 99% contaminating the ecosystem and also creating problems of resistance and resurgence. Sabry *et al*. (2010) reports that when viricidal chemicals are applied for use in plant disease management, they will affect both the host plant and the pathogen. Viruses depend on its host to survive and multiply and viral diseases cannot be cured however plant viruses can be managed. Natural products from plants or botanicals have been found to be good inhibitors of plant viruses (Upadhyay, 2012). One of such natural products are lattices from plants. A botanical pesticide is a type of biopesticide made up of crude plant extracts or purified compounds of plant species for managing pests and diseases in an agro-ecosystem. Lattices are natural plant polymers, emulsion like sticky substance exuded from various plant parts after having a small tissue injury. They can also be described as a complex

mixture of proteins, alkaloids, starch, sugars, oils, tannins, resins and gums (Hagel *et al*, 2008: Pickard, 2008: Thomas *et al*, 2008). Latex has been known to play an important role in plant-insect interactions because it contains cysteine proteases which provide defense against herbivores insects (Kitajima *et al*. 2010).

The Moroccan watermelon mosaic virus (MWMV) is a potyvirus commonly transmitted in a non-persistent manner by aphids (Owolabi and Ekpiken, 2014). Vegetables and particularly the Curcubits have been known to be infected by this virus causing considerable damage leading to losses running into billions of dollars (Lecoq, 2004) and they induce symptoms such as lesions on *Chenopodium sp*. and mosaic and leaf malformation in susceptible curcubits host (Malandraki *et al*., 2014). The use of synthetic chemicals for disease management often results in residuals effects after use which eventually causes soil pollution. Also, synthetic chemicals are known not to be bio-degradable therefore the use of latex for disease management will solve the problem of pollution and at the same time manage the disease. Plants derived compounds are considered the best in the management of viral diseases of crop plants. Hansen (1989) listed features of antiviral compounds to include solubility in water or non-phytotoxic solvents; being effective against agriculturally important viruses at non-phytotoxic concentrations and that it being easily taken up by plants. Jeyalakshmi *et al*, (2015) reports that antiphytoviral compounds affects the development of viral diseases in plants by either inactivating the viral pathogen or by indirectly inducing host resistance (Mahdy *et al*. 2007). The potyviruses require cysteine proteases for their multiplication and propagation, therefore plants having cysteine proteases inhibitors will resist the growth of such viruses.

Sabry *et al*. (2010) reported that the cysteine protease inhibitors present in the latex of Ficus effectively inhibited tobacco necrosis virus (TNV) infection, when the leaves of *Phaseolus vulgaris* were inoculated with a mixture of TNV and latex protein. In this study plant lattices from *Jatropha curcas* (Euphorbiaceae), *Thevetia neriifolia* (Apocynaceae) and *Carica papaya* (Caricaceae) will be considered.

2. MATERIALS AND METHODS

2.1 Latex collection

Latex was collected from healthy plants of *C. papaya*, *T. neriifolia* and *J. curcas*. These plants were sourced from the Cross River University of Technology teaching and research garden, Calabar. The extraction of the latex was achieved by making incisions on the stem of young plants by using a sterile scalpel or by cutting the leaves from the petiole and collecting them with a sterile specimen bottle (Freitas *et al.* 2007). After collection, the latex was stored in a refrigerator at 4^{o}C. Before the latex is used, it is centrifuged for 5 minutes at 5000 rpm so that tissue debris from latex collection is removed and the supernatant obtained is suitably diluted for use.

2.2 Virus culturing

The *Moroccan watermelon mosaic virus* (MWMV) was sourced and isolated from infected *Lagenaria breviflora* in the teaching and research botanic garden of the University of Calabar. They were maintained and propagated

by serial inoculations on *Cucumis sativa* in the screenhouse as described by Anfoka and Buchenauer, 1997. The inoculum of MWMV was prepared by grinding 1 gram of young symptomatic leaves in 0.05 M potassium phosphate buffer, pH 8.0 in a pre-cooled oven sterilized pestle and mortar. The resulting pulp after the grinding is passed through layers of cheesecloth and centrifuged at 3000 rpm for 5 minutes. The supernatant obtained is then diluted with buffer to be used for inoculation purposes.

2.3 Preliminary studies

Leaves of the second test plant (*Chenopodium sp.*) were dusted with carborundum and then an equal volume of the inoculum of MWMV and latex from *J. curcas*, *C. papaya* and *T. neriifolia* respectively were mechanically transferred on to the upper surface of young healthy leaves of the test plant using a glass spatula. After 10 to 14 days, number of local lesions on the leaves are examined and counted as a confirmation of infection by the virus. It was replicated three times. Control test for the inhibitory properties of the latex from each of the plants was achieved by inoculating with equal volumes of the MWMV inoculum and distilled water.

2.4 Inhibition test.

The leaves of *Chenopodium sp*. were dusted with the abrasive silicon carbide (carborundum) and then rubbed with mixtures of 1µg/ml concentration of MWMV and varying concentrations of latex (0.25, 0.50, 0.75 and 1.0 mg/ml) from *C. papaya* after the mixture of MWMV and latex have been kept for 15 minutes. Observation was made after 10 to 14 days for symptoms development of infection.

A similar test was repeated using latex from *J. curcas* and applying same concentrations.

2.5 Effect of latex on MWMV transmission by *Aphis spiraecola*

Aphis spiraecola was collected from the field, reared in screen cages, maintained on a virus-free *Cucumis sativa* and kept to be used for transmission studies. When the transmission test is to be carried out, the aphids are starved for an hour before transferred to an infected plant for acquisition feeding. They are then transferred to a healthy *C. mannii* plant that had its' leaves previously rubbed with the latex from *C. papaya* (1 mg/ml) for inoculation feeding. Feeding by the aphids takes seconds to a few minutes to be accomplished. A total of 20 plant stands were set up with each stand having 3 aphids for transmission test and three replications.The above test was repeated using *J. curcas* for transmission studies. The control for the test is repeating the process without introducing latex.

2.6 Statistical analysis

The experimental design adopted in this study was the complete randomized design. The preliminary studies to ascertain the effectiveness of the latex was analyzed using univariate analysis. Similarly, the results for MWMV inhibition during transmission by aphids using plant latex was analyzed by employing a 2-factor (latex type and latex condition) univariate analysis of variance while the minimum inhibition concentration of the latex was also analyzed using a 3-factor (inoculations, plants and latex concentrations) univariate analysis of variance.

3. RESULTS

3.1 Preliminary studies

Table (1) presents the results for the preliminary test of the lattices of *J. curcas, T. neriifolia* and *C. papaya* on MWMV. It indicates that the latex from *C. papaya* gave the highest inhibition (94.29%) of the virus followed by *J. curcas* (71.50%) and lastly by *T. neriifolia* with 15.60%. The inhibitions from the lattices of *C. papaya* and *J. curcas* were significant ($P \leq 0.05$) against MWMV as compared to *T. neriifolia* leading to their further use in subsequent experiments. When multiple comparisons of the means of *J. curcas, T. neriifolia* and *C. papaya* were made using Least Square Difference (LSD), the means between *C. papaya* and *J. curcas* wasfound not to be significant ($P \geq 0.05$), however when both means were compared to that of *T. neriifolia*, they were 16.5 and 13.0 respectively and were highly significant ($P \leq 0.05$). Also when the estimated marginal means of lesions was plotted against inoculation with latex and against inoculation with water, it showed an interaction between the lattices of the plants and inoculation (Figure 1).

3.2 Minimum inhibition concentration of the latex of *C. papaya* and *J. curcas* on MWMV.

The results in tables (2a) and (2b) showed a general pattern of an increase in percentage inhibition as the concentration of the latex is also increased from 0.25 mg/ml to 1mg/ml. The latex of *C. papaya* caused inhibition to increase from 73.70% at 0.25 mg/ml to complete inhibition of 100% at 1.0 mg/ml. Also the latex of *J. curcas* increased inhibition from 61.45% at 0.25 mg/ml to 81.02% at 1.0 mg/ml. The analysis of variance for the test of minimum inhibition concentration of the lattices revealed that there was an interaction of the marginal means of inoculations, plants and latex concentrations when inoculations was done with and without latex and they were all found to be significant ($P \leq 0.05$). The relationship between these three factors for *J. curcas* and *C. papaya* are shown in the figures (2a) and (2b) below.

When the means of the latex concentrations were compared as they affected number of lesions produced, the mean difference between the concentrations of 0.25 and 0.50 mg/ml was found not to be significant ($P \geq 0.05$), however when same (0.25 mg/ml) was considered against 0.75 mg/ml and 1.00 mg/ml they were significant ($P \leq 0.05$).

3.3 Effect of latex on transmission efficiency of MWMV

Tables (3a) and (3b) indicated that the efficiency of the transmission of MWMV by *Aphis spiraecola* was affected. The percentage infected in the transmission studies when the latex of *C. papaya* was employed as an inhibiting agent revealed that it declined from 100% when transmission was carried out without latex to as low as 10% when latex was mixed with MWMV. When the latex of *J. curcas* was employed in place of *C. papaya*, there was a reduction of transmission efficiency of MWMV by the aphid from 100% when transmission was carried out without the latex to as low as 25 % when done with the latex. When the means of transmission efficiency is plotted against the latex condition (with latex/ without latex), it revealed an interaction between these parameters.

4. DISCUSSION

The results of the experiments presented above shows that the lattices from *C. papaya* and *J. curcas* are capable of inhibiting MWMV, thus a

veritable source for the management of diseases caused by the plant virus. Tewari and Shukla (1982) reported that lattices from ten selected plants inhibited Watermelon mosaic virus (WMV). Also, Caffini *et al.* (1988) reported the complete inhibition of Sunnhemp mosaic virus and Tobacco mosaic virus (TMV) by *Calotropis procera* and *Carica papaya* respectively. The latex of *J. curcas* and *C. papaya* were found to be significantly effective against MWMV than the latex of *T. neriifolia,* these could be argued to be as a result of the presence of cysteine proteases in *J. curcas* (curcain) and *C. papaya* (papain). Jeyalakshmi *et al.* (2015) opined that potyviruses, comoviruses, closteroviruses, nepoviruses and tymoviruses require cysteine proteases for their multiplication and propagation, therefore plants possessing cysteine protease inhibitors resist the growth of such viruses. These inhibitions are achieved through the inactivation of virus particles by ribosomal inactivating proteins (RIPs) present in antiviral principles (AVPs) from botanicals and inactivation of virus activity are through the activity of these enzymes – N-glycosidase, RNase, DNase, Phospholipase and superoxide dismutase (Wood, 1982; Sharma *et al*. 2004; Choudhary *et al*. 2008). Nielson and Boston (2001) reports that RIPs inactivate ribosomes by depurinating rRNA in a highly conserved stem-loop structure in the 28s RNA, thereby blocking its further participation in protein synthesis. Sabry *et al.* (2010) suggested that the mechanism by which the lattices from plant function is that they conjugate with the protein virus in order to disintegrate them.

It was also observed that for either of the latex, as little as 0.25 mg/ml was capable of inhibiting as much as 60% of the virus. Furthermore, apart from the inhibitory property of the latex against plant virus, the latex is also known to play a role in plant-insect interactions which could serve as a deterrent on insects perching on plants. Plant latex have been reported to contain defense protein and cardenoides. Latex also possess the enzyme chitinases which can affect insect skin thereby deterring virus transmission (Diaz-Perales *et al.* 1998; van Loon *et al.* 2006; Wasano *et al.* 2009).

CONCLUSION

This study has highlighted that latex from botanicals can inhibit plant viruses by inducing an antiviral state at the site of the application (local resistance) or through induced systemic resistance.

The study also showed that plant latex from the three plants were capable of inhibiting the Moroccan watermelon mosaic virus albeit with varying abilities. The future of plant virus management certainly lies with the use of botanicals and its wide range of advantages over synthetics therefore further studies will be needed to investigate more lattices found locally and its formulation into cheap and affordable pesticides that will be used in the management of plant viruses.

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Table 1: Preliminary potency test results of the three lattices

	Inocula + Latex	Inocula + Water	% Inhibition
Jatropha curcas	18	63	71.50
Carica papaya	4	**70**	94.26
Thevetia neriifolia	49	58	15.60

All values are means of three replications.

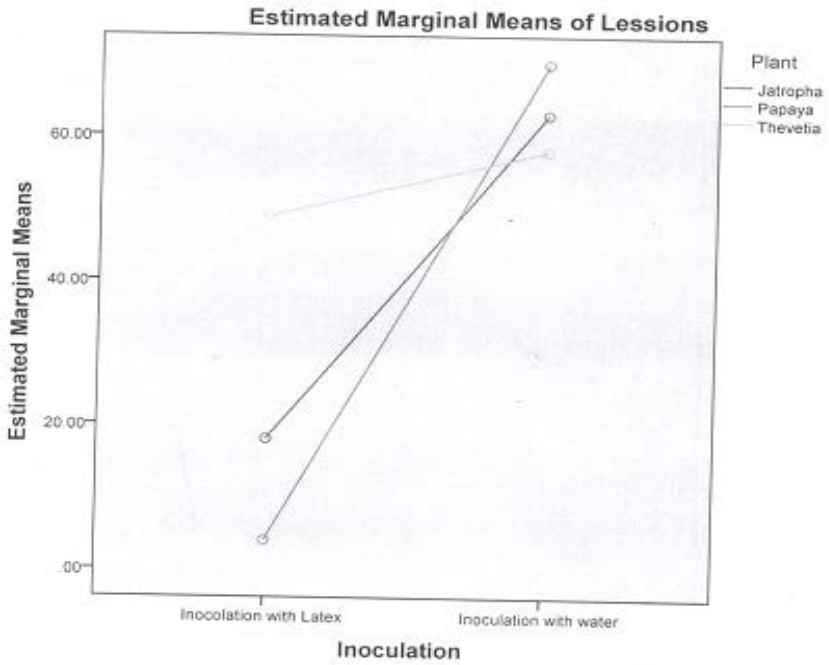

Figure 1**:** Mean number of lesions in response to lattices of *Carica papaya, Jatropha curcas and Thevetia neriifolia*

Table 2a: Minimum inhibition concentration of the latex of *Carica papaya* on MWMV at different concentrations

Latex concentration	Number of Lesions (count)		
	With Latex	Without Water	% Inhibition (mg/g)
0.25	20	76	73.76
0.50	15	80	81.25
0.75	5	89	94.39
1.00	-	96	100.00

Values of number of local lesions are means after three replications

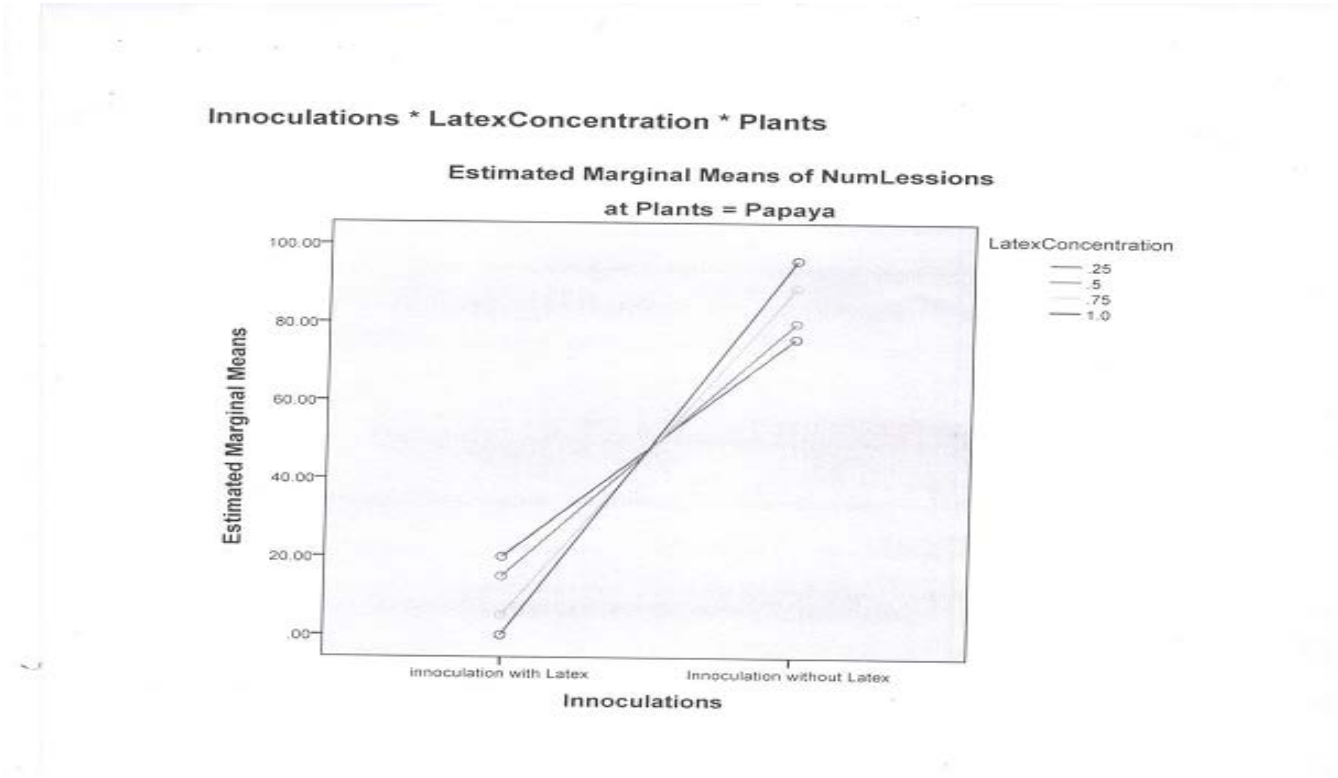

Figure 2a: Marginal means of number of lesions with and without *Carica papaya* latex.

Table 2b: Minimum inhibition concentration of the latex of *Jatropha curcas* on MWMV at different concentrations

Latex concentration	Number of Lesions (count)		
	With Latex	Without Water	% Inhibition (mg/g)
0.25	32	83	61.45
0.50	27	87	68.97
0.75	19	80	76.25
1.00	15	79	81.02

Values of number of local lesions are means after three replications

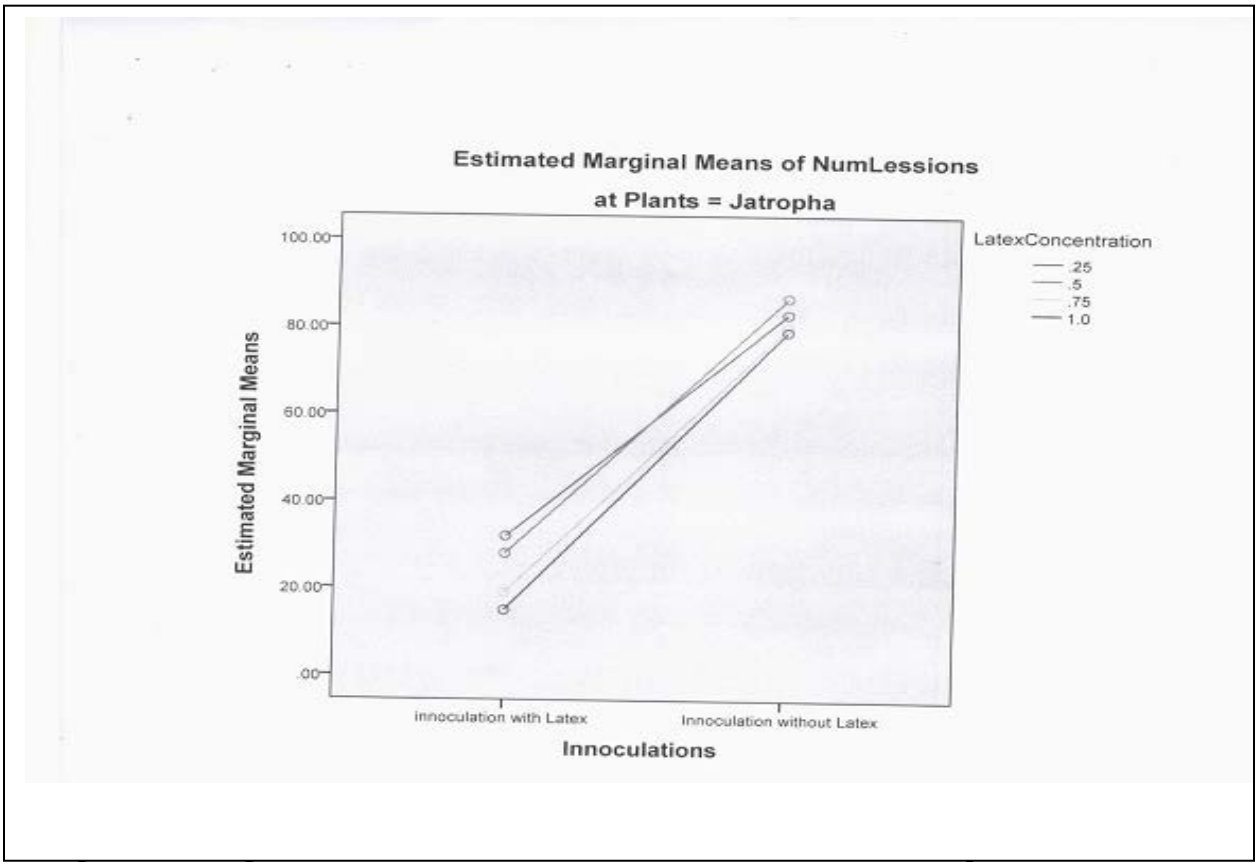

Table 3a: Effect of *Carica papaya* latex on transmission of MWMV by *Aphis spiraecola*

Replications	With Latex		Without Water	
	No. of infected/inoculated	% infected	No. of infected/inoculated	% infected

	plants		plants	
1	20/20	100	3/20	15
2	18/20	90	2/20	10
3	20/20	100	3/20	15

- Aphid (three/plant) fed on MWMV infected plants, then transferred to healthy plants for inoculation feeding that has been treated with/without latex.

Table 3b: Effect of *Jatropha curcas* latex on transmission of MWMV by *Aphis spiraecola*

Replications	With Latex		Without Water	
	No. of infected/inoculated plants	% infected	No. of infected/inoculated plants	% infected
1	19/20	95	5/20	25
2	19/20	95	7/20	35
3	20/20	100	6/20	30

- Aphid (three/plant) fed on MWMV infected plants, then transferred to healthy plants for inoculation feeding that has been treated with/without latex.

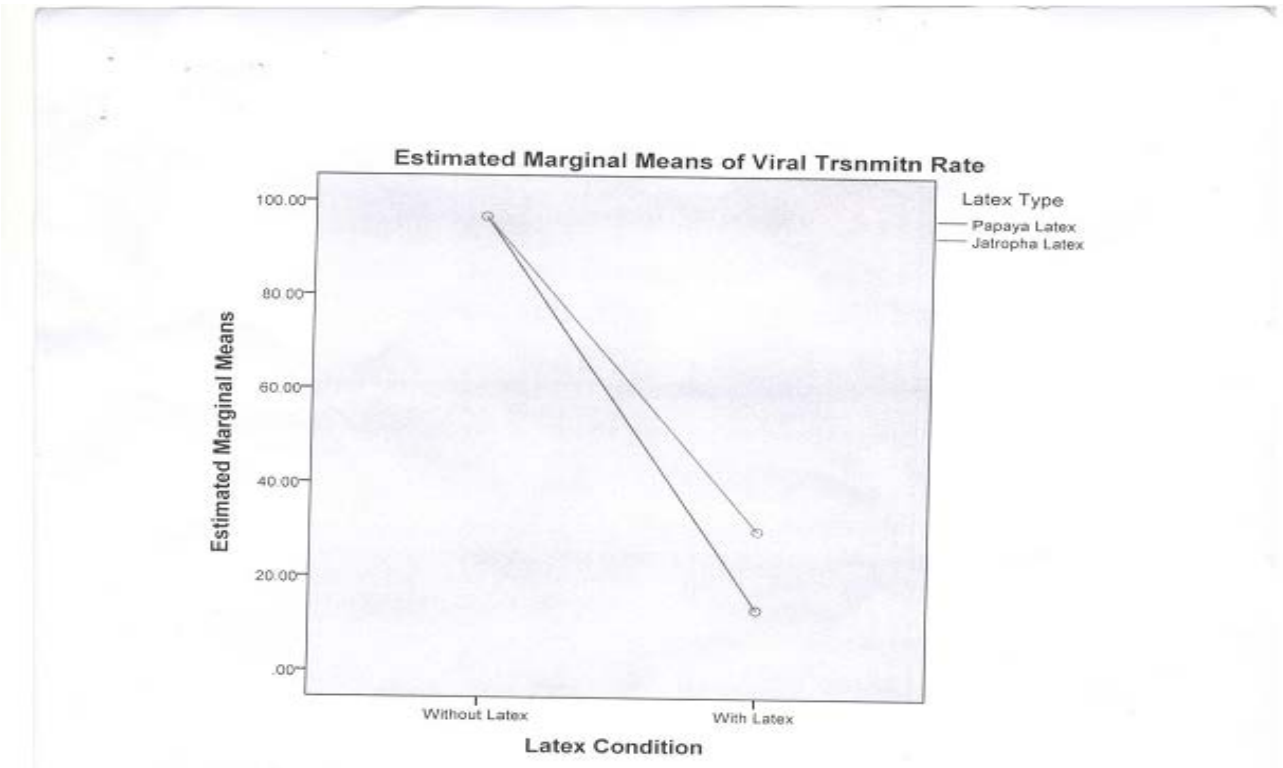

Figure 3: Marginal means of MWMV transmission rate by *Aphis spiraecola* with and without the latex of *Carica papaya* and *Jatropha curcas*

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