

Mixed Viral Infection and Growth Stage on Chilli (*Capsicum annuum* L.) Production

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ABSTRACT

The objective of this research was to study the effects of mixed viral infection and growth stage on chilli (*Capsicum annuum* L.) production. This study was carried out in a split plot design with plant stage as the main plot and viral infection as the sub-plot. Plant stage as the main plot consisted of four levels, i.e 15 days, after transplanting (DAT), 40 DAT, 65 DAT, and 90 DAT, whereas viral infection as sub-plot consisted of 5 inoculation of viruses, i.e., no viral infection (control), cucumber mosaic virus (CMV) + tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) + potato virus Y (PVY), CMV+PVY and CMV+PVY+TMV. Each treatment was replicated five times. The inoculations were conducted mechanically by rubbing single young leaves which had been dusted with carborandum 400 mesh, with inoculum of respective viruses. Parameters observed in this research were plant height, biomass, and chillies production (number of fruits and the weight of total fruits yield) for each plant. Results of the study showed that that growth stage and viruses significantly affect the plant height and yield components of chilli. Mixed viral infection among CMV, PVY and TMV caused a significant reduction in the chilli biomass and production. Although viral infection increased the plant height, the infected chilli seemed unhealthy. There existed interaction effects of mixed viral infection and growth stages on the chilli biomass. All viral infection and growth stages reduced significantly the biomass of the chilli, with the lowest found at the mixed viral infection of TMV+PVY (18.5%) and the highest was at CMV+TMV (44%). Double mixed infection of CMV+TMV and CMV+PVY caused 52 and 49% reduction of both the total number of fruits and total weight of fruits/plant respectively being the highest reduction compared to other treatments.

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INTRODUCTION

Chilli (*Capsicum annuum* L.) production in Indonesia cannot fulfil the national needs for chilli, forcing the Government of Indonesia to import chilli of up to 16,000 tonnes per annum (MOA, 2009). On average, chilli production in Indonesia is 4.35 tonnes per hectare, the amount which is far from potential production, that is, 10 tons per hectare. One constraint hampering the chilli production in Indonesia and other countries is viral disease (Suryaningsih *et al.*, 1996). In Indonesia, under certain condition the infection of viral diseases can reach more than 90 percent of the chilli production area (Duriat, 1996).

Viral diseases on chilli are considered as the main limiting factor in the chilli cultivation, especially in Indonesia. In Asia, at least 35 viruses are known to infect the chilli plantation and the most important viruses are cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), potato virus Y (PVY). Mixed infection of the viruses can cause more severe diseases on the pepper plant (Arogundade *et al.*, 2012). At least ten different viruses have been reported to infect a number of chilli cultivars in Indonesia (Duriat, 1996; Suryaningsih, Sutarya, & Duriat, 1996). Four of them are cucumber mosaic virus (CMV), chilli veinal mottle virus (ChiVMV), potato virus Y (PVY) and tobacco mosaic virus (TMV), all of which can induce mosaic symptoms (Nurdin, 1998; Sulandari, 2004). Virus diseases caused yield loss in the range of 60-100% and these were considered the major constraints to the

economic production of the crop (Green, 1993; Subekti, 2005).

Experts have noted that there exist around 13-35 viruses which attack chilli plants in the chilli plantation of tropical and sub-tropical regions. Prevalence of viral diseases from time to time have changed, as shown by the research results of Research Centre for Vegetable Crops in Lembang Bandung West Java, Indonesia, between 1995 and 1996 (Duriat & Gunaini, 2003). Taufik *et al.* (2005) stated that CMV, PVY and TMV have spread throughout most of the chilli crops in Indonesia. A very recent study in South Sulawesi by Taufik *et al.* (2011) reported that CMV, together with TMV, infects pepper plants.

The objective of this research was to study the effects of mixed infection between TMV, CMV and PVY and growth stage on chilli production. Such information is very important in determining the proper means of controlling the diseases caused by viruses in the chilli production.

MATERIALS AND METHODS

This research was carried out in a green house at Bukit Lama Palembang Indonesia between January and August 2011 using a split plot design with plant stage as the main plot and viral infection as the sub-plot. Plant stage as main plot consisted of four levels, i.e. 15 days after transplanting (DAT), 40 DAT, 65 DAT, and 90 DAT, whereas viral infection as sub-plot consisted of 5 inoculation of viruses, i.e. no viral infection (control), CMV+TMV, CMV+PVY, CMV+PVY

and CMV+PYV+TMV. Each treatment was replicated five times. Local hot chilli seedling (15 days old) were transplanted onto polybags containing 10 kg of sterile soil mix (top soil:organic soil = 2:1). The inoculations were conducted mechanically by rubbing single young leaves which had been dusted with carborandum 400 mesh, with inoculum of respective viruses.

The parameters observed in this study were plant height, biomass, and chillies production (number of fruits and the weight of total fruits yield) by the each plant. The data were analyzed to determine the effects of virus on vegetative growth and yield using the analysis of variance (ANOVA), with the Duncan's Multiple Range Test (DMRT) comparison among means (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Analysis result of the effects of the overall treatments in the study showed that growth stage and viruses singly significantly affected the plant height and yield components of chilli. The interaction effect of the chilli growth stage and viral infection significantly

affected the plant height, average weight of fruit and biomass of the tested chilli plants. However, there was no interaction effect of growth stage and viral infection on the number of fruits and weight of fruits (Table 1).

The results of further test showed that the growth stage at the time of viral inoculation significantly affected the plant height (Table 2). Based on the test for a number of viral inoculation on the four growth stages 15 DAT, 40 DAT and 90 DAT, there existed a number of viral treatment which increased significantly the plant height. Relative values (percentage towards control) of the plant height at 15 DAT ranging from 78.9-119.7%, compared with the relative values of 40 DAT (98.5-149.3%) and 90 DAT (105.3-132.9%).

In Table 2, it was clear that CMV+TMV resulted in the highest plant heights of chilli at 15 and 40 DAT, while at 90 DAT the highest plant height was at CMV+PYV+TMV. However, all inoculated chilli plants in this study, which had relatively higher plant height seemed to be unhealthy compared to the control (see Fig.1). This result is

TABLE 1
Summary of the effects of viral infection and growth stage on the plant height and chilli production

No	Observed variables	Treatment		
		Growth stage	Viral infection	growth stage*Virus
1	Plant height	**	**	**
2	Number of fruits	**	**	ns
3	Weight of fruits	**	**	ns
4	Average weight of fruit	**	**	*
5	Biomass	**	**	*

** and * = significantly different at $p < 0.01$ and ≤ 0.05 , respectively
ns = not significantly different

not surprising for other researchers such as Kusumawati, Hadiastono and Martosudiro (2013) who reported that viral attack such as TMV on various growth stage can suppress plant growth and decrease cayenne pepper production.

Further test (Table 3) showed that there existed interaction effects of mixed viral infection and growth stages on the chilli biomass. All double and triple viral infection significantly reduced biomass regardless of the growth stage of the chilli plant. Relative values of the biomass at 15 DAT had reduction ranging from 64.9-81.5%, followed by those of 40 DAT ranging from 65.2-71.5%, and those of 65 DAT ranging from 60-64.1% compared with control (no viral treatment). Surprisingly, relative values of biomass at 90 DAT are highest which is ranging from 46.1-78% compared with the control. The lowest reduction of chilli

biomass was at the mixed viral infection of TMV+PYV (18.5%) and the highest was at CMV+TMV (44%). Reduction of 58% of cayenne pepper biomass due to viral attack by TMV was reported by Kusumawati, Hadiastono and Martosudiro (2013). Meanwhile, Gallitelli (1998) and Suryaningrat *et al.* (1996) have reported the presence of CMV, together with other viruses in chilli plantation, which has caused big losses in chili yield.

The amount of reduction in chilli production in the study was clearly seen in further test. The results of the test (Table 4) showed that the effect of mixed viral infection among CMV, TMV, PYV caused a significant reduction in the chilli production. Double mixed infection of CMV+TMV and CMV+PYV caused 52 and 49% reductions of both the total number of fruits and total weight of fruits/plant, respectively. These



Fig. 1: Infection symptoms of the viruses attack on leaves at 90 DAT (from leaf to right – control, CMV+TMV, CMV+PYV, TMV+PYV, CMV+TMV+PYV)

were also found as the highest reductions compared to the other treatments. The mixed infection of plant viruses that has caused severe symptoms showed that interaction among viruses occurs within plant tissues (Kosaka & Funishi, 1997; Zhang *et al.*, 2001). The interaction between two or more of virus pathogen can occur either synergistic, additive or antagonism (Oku, 1994; Matthew, 1991). A study by Akin and Nurdin (2003) reported a reduction in their chilli yield ranging 21-67% due to TMV attack. They also added that TMV inhibited generative growth in the chilli crop.

In Table 4, the growth stage is shown to cause significant reduction in chilli yields, i.e. number of fruits per plant and total weight of fruits per plant. The growth stages of 15 DAT, 65 DAT and 90 DAT reduced significantly the number of fruits per plant, which ranged from 42 to 38% compared to the control. Growth stage also reduced significantly the total weight of fruits per plant. Growth stage of 65 DAT, followed by 90 DAT and 15 DAT caused reductions in the total weight of fruits per plant, which ranged from 46 to 31.5% compared to the control. A study conducted by Kusumawati,

TABLE 2

The interaction effects of mixed viral Inoculations and growth stages of the chilli on plant height (cm)

Viral treatment	Days after transplanting (DAT)								
	15		40		65		90		
No virus	71	ab	69	a	79	a	85	a	
CMV+TMV	56	(78.9)	a	72	(104.3)	a	71	(89.9)	a
CMV+PYV	85	(119.7)	b	103	(149.3)	b	77	(97.5)	a
TMV+PYV	59	(83)	a	82	(118.8)	a	71	(89.9)	a
CMV+PYV+TMV	65.5	(92.2)	a	68	(98.5)	a	65	(82.3)	a
Average	67.3	(93.7)		78.8	(117.8)		72.6	(89.9)	
							95.7	(115.85)	

Figures in a column that are followed with the same letters mean there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

TABLE 3

The interaction effects of mixed viral inoculations and growth stages of the chilli on biomass (g)

Viral treatment	Days after transplanting (DAT)								
	15		40		65		90		
No virus	157	b	214	b	170	b	178	c	
CMV+TMV	108	(68.8)	a	139.5	(65.2)	a	108	(63.5)	a
CMV+PYV	102	(64.9)	a	153	(71.5)	a	109	(64.1)	a
TMV+PYV	128	(81.5)	a	136	(63.5)	a	102	(60)	a
CMV+PYV+TMV	119	(75.8)	a	134	(62.6)	a	102	(60)	a
Average	122.8	(72.72)		155.3	(65.7)		118	(61.9)	
							103	(61.5)	

Figures in a column that are followed by the same letters mean that there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

Hadiastono and Martosudiro (2013) was in line with this study's findings, whereby growth stage at the time of viral inoculation reduced cayenne pepper significantly.

An interaction effect of viral infection and growth stage was found on the average weight of fruit. Mixed viral infection of CMV+PYV at 15 DAT reduced the average weight of fruit around 30% compared to the control. In contrast, the mixed viral

infection of CMV+TMV, CMV+PYV and TMV+PYV increased the average weight of the chilli fruit. This is not surprising for the chilli's total number of fruits per plant and total weight of fruits per plant for the treatments, which were lower than the control. It is believed that the rotten fruits are due to viruses containing more water compared to healthier plants (the control) (see Table 5). Aeni (2007) reported that the

TABLE 4
The effects of mixed viral inoculations and growth stages on chilli production

Factors tested	Total number of fruits per plants		Total weight of fruits/plant (g)	
Viral treatment				
No virus	53	b	238	c
CMV+TMV	24 (48)	a	115 (48.3)	a
CMV+PYV	25.5 (51)	a	123 (51.7)	a
CMV+PYV+TMV	43 (81)	a	132 (55.5)	ab
PYV+TMV	53 (100)	b	173 (72.7)	b
Growth stage				
15 DAT	33 (62)	a	163 (68.5)	ab
40 DAT	47 (88.7)	b	179.5 (75.4)	b
65 DAT	29 (54.7)	a	129 (54)	a
90 DAT	28 (52.8)	a	143 (60)	ab

Figures in a column that are followed by the same letters mean that there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

TABLE 5
The interaction effects of mixed viral inoculations and growth stages of the chilli on the average weight of fruit (g)

Viral treatment	Days after transplanting (DAT)							
	15		40		65		90	
No virus	5.1	b	4	ab	4.8	a	4.4	a
CMV+TMV	5.3 (104)	b	3.5 (90)	a	4.7 (97.9)	a	6.6 (150)	b
CMV+PYV	3.8 (70)	a	5.3 (132)	b	5.3 (110.4)	a	5.8 (132)	b
TMV+PYV	4.8 (130)	ab	3.4 (85)	a	4 (83.3)	a	6.2 (140)	b
CMV+PYV+TMV	5.3 (110)	b	3.8 (110)	a	4.5 (93.7)	a	5.5 (129.5)	ab
Average	3.86 (110)		4 (125.5)		4.6 (96.3)		5.7 (137.9)	

Figures in a column that are followed by the same letters mean there is no difference at $p \leq 0.05$ DMRT. Figures in brackets are the relative percentage values to the control.

nett assimilation of the affected chilli plants by viruses was much lower than that of the healthy ones causing lower production of the chilli crop.

The low chilli production from the treatment of mixed TMV with other viruses is clearly related to the attack symptom shown by every virus or their combination (Sutarya, 1991). The yield reduction happened to all viral treatments. However, the highest reduction was for the CMV+PYV treatment, followed by other mixed viral treatments. The least resistance of chilli growth stage was by the time chilli having its first flowers. At this stage, the chilli plants are in need of more nutrition on the one hand, and experiencing metabolism disturbance on the other. In this situation, the plants suffered heavy stress. The plants that were attacked by viruses experienced heavy chlorosis in their leaves as the viruses disturb the enzym anaplerotic, which lower the rate of the plants photosynthesis rate (Fanayama & Terashima, 2006). This is in agreement with a report by Goodman, Kiraly and Zaitin (1967). Akin and Nurdin (2003), Gallitelli (1998), and Suryaningsi *et al.* (1996) have reported big losses in chilli yields as a result of CMV and TMV attacks in their chilli crops.

CONCLUSION

From this research, it can be concluded that growth stage and viruses significantly affect the plant height and yield components of chilli. In specific, the mixed viral infection among CMV, PYV, and TMV caused significant reductions in the biomass and

chilli production. Although viral infection increased the plant height, the infected chilli seemed unhealthy. The interaction effects of mixed viral infection and growth stages were found on the chilli biomass. All viral infection and growth stages reduced significantly the biomass of the chilli, with the lowest reduction at the mixed viral infection of TMV+PYV (18.5%) and the highest at CMV+TMV (44%). Double mixed infection of CMV+TMV and CMV+PYV caused 52 and 49% reductions of both the total number of fruits and total weight of fruits/plant, respectively, being the highest reductions compared to the other treatments.

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