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An appropriate method to determine the interaction type of Cucumber mosaic virus (CMV) and Bean yellow mosaic virus (BYMV)

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ABSTRACT- The occurrence of viral co-infection is a common phenomenon in cultivated and native plant species and can alter the dynamics of virus infection. In this study, disease progress was examined in single and mixed infections of Cucumber mosaic virus (CMV) and Bean yellow mosaic virus (BYMV) by measuring the rate of symptom development, disease severity and area under disease progress curve on infected bean and broad bean. Simultaneous infection of bean to CMV and BYMV caused higher disease severity; however, no significant differences in disease severity were found on broad bean. In this study, a novel statistical approach (Abbott's approach) was used to recognize virus joint action in bean and broad bean hosts. Abbott's approach indicated synergistic effect between CMV and BYMV on bean only while the interaction was antagonistic when growth responses were considered on the same host. In broad bean plants inoculated with CMV+BYMV, CMV and BYMV, the two viruses affected disease severity and growth responses in an additive manner. Taken together, Abbott's approach was an appropriate method to determine synergistic interaction in these pathosystems.

INTRODUCTION

Legumes including broad bean (*Vicia faba*) and common bean (*Phaseolus vulgaris*) are considered as main sources of protein and minerals in human diets (Shellie-Dessert and Bliss, 1991). Bean yellow mosaic virus (BYMV) and Cucumber mosaic virus (CMV) are two important plant viruses causing considerable economic losses to legumes (Hemida, 2005; Shah et al., 2006; Taylor and Shail, 2006). Mixed infection of plants with two or more viruses is a common phenomenon in nature and the co-infecting viruses can change the dynamics of infections and epidemiology of viruses (García-Cano et al., 2006; Hacker and Fowler, 2000; Malapi-Nelson et al., 2009; Martin and Elena, 2009; Sánchez-Navarro et al., 2006; Wintermantel et al., 2008).

The interactions between plant viruses in multiple infections including antagonism or synergism may have significant impacts on epidemiology and management of the plant viral diseases (Hull, 2014). In antagonistic interactions, the viral infection in mixed infections is reduced and one virus restricts another virus (Syller, 2016) while in synergistic interactions, viral symptoms and titers of both viruses are enhanced in mixed infections (García-Cano et al., 2006).

In addition to change in viral accumulation and symptoms severity, growth parameters such as plant height and fresh weight of plant organs have also been used to study the expression pattern of synergism between the viruses (Murphy and Bowen, 2006; Wintermantel, 2005). It has been shown that single and mixed infections of CMV and BYMV are common in Iranian leguminous fields. The average infection rate of CMV, BYMV and their mixed infection in four provinces (Tehran, Ghazvin, Markazi and Gilan) was estimated to be 11.57, 10.65 and 6.48 percent, respectively (Tahmasebi et al., 2010). These two viruses belong to different virus groups with distinct genome organizations. CMV (genus *Cucumovirus*) has positive-sense RNA genome encoded five proteins by three genomic and two subgenomic RNAs (Ding et al., 1994; Palukaitis et al., 1992) while BYMV (genus *Potyvirus*) has monopartite positive sense-RNA genome encoded 9 to 10 proteins (Revers et al., 1999). Most attention in virology research has traditionally been given to the properties of individual virus species whereas comparatively little attention has been paid to within-host interactions between viruses or between viruses and microorganisms in multiple infections (Lidsky et al., 2009; Rentera-Canett et al., 2011).

The type of interaction (synergism/antagonism) between the two viruses may be estimated by comparing the observed versus expected efficacy of their mixture (known as synergy ratio) (Murphy and Bowen, 2006). Interaction may also be assessed by comparing the

observed versus expected doses that provide the same level of effect, what is referred to as co-toxicity ratio in the context of joint action of mixtures (Cedergreen, 2014). The empirical approach for determining synergy could be based on either the additive dose model (ADM) or the multiplicative survival model (MSM). The ADM and MSM correspond to cases of similar and different joint action of mixtures, respectively (Morse, 1978). Accordingly, information or assumptions about the biological mode of action of the components in the mixture are required to verify if the components of a mixture affect the same or different system(s) and also to distinguish between ADM and MSM. If the action of components is assumed to be similar then Wadley method (Wadley, 1945) can be used and when there is no assumption about the form of dose response curves of the components, the ADM could be used. In both cases, dose response curves of the mixture and its components are required. If different actions for the components of the mixture are assumed, then Abbott procedure is considered as a basic approach for verification of synergy (Kosman and Cohen, 1996).

The type of interaction in mixed-infection of plant viruses can explain the effects of mixed viral infection on disease development and crop losses. In this study, a novel statistical and theoretical approach was used to recognize virus joint action in two important legume crops. Accordingly, the aim of this study was to determine the type of interaction in mixed-infection of CMV and BYMV according to Abbott procedure.

MATERIALS AND METHODS

Sources of Viruses and Plant Materials

CMV and BYMV isolates were obtained from naturally infected bean plants, collected from a field located in Khomein, central region of Iran. Viral isolates were purified biologically by serial local lesion passages on *Chenopodium quinoa* and purified viruses were subsequently propagated and maintained on *P. vulgaris*. Seeds of broad bean (*Vicia faba*) cultivar (cv) Lahijan and common bean (*P. vulgaris*) cv. Bountiful were surface sterilized by soaking in 0.5% sodium hypochlorite for five minutes and were planted in six-inch pots filled with a soil mix consisting of equal proportions of sand and soil. Experimental pots were kept in a glasshouse set at 27±3°C with 30-50% relative humidity and 16:8 light:dark photoperiod.

Virus Treatments

Plants of bean and broad bean (20 days old) were mechanically inoculated with CMV, BYMV and CMV+ BYMV. Viral inocula were prepared by grinding leaves of CMV- or BYMV-infected bean or broad bean leaf tissues in 0.1 M phosphate buffer (PB), pH 7 (1:10 W/V). For mixed inoculation of CMV+BYMV, saps from both bean and broad bean plants infected with either virus were mixed in a 1:1 (V/V) ratio

immediately prior to inoculation (Taiwo et al., 2007). The experiment was laid out in a factorial arrangement with two factors; host plant (with two levels of bean and broad bean) and inoculum (CMV or BYMV, mixed CMV and BYMV and mock inoculated plants as control) using completely randomized design as the basic design. Plant growth parameters were measured on individual plants grown in plastic pots. The number of replicates for evaluating plant growth parameters and disease severity were 30 bean and 40 broad bean plants arranged in 6 and 8 pots, respectively. Each pot contained five plants for evaluation of disease severity ratings.

Disease Severity

Disease symptoms were scored on an ordinal scale with 5 classes from 0 = symptomless to 4=severe mosaic (Table 1) at 12, 16, 23, 30, and 36 days post inoculation (dpi) for bean and 5, 7, 10, 16, 22, 28, and 34 dpi for broad bean. Plant height and fresh weigh were evaluated on individual bean or broad bean plants at 36 dpi. Stem length from soil level to stem tip was used as a measure of plant height 36 dpi. Infection status of inoculated plants was checked by double-antibody sandwich enzyme-linked immunesorbent assay (DAS-ELISA) (Clark and Adams, 1977) using polyclonal antibodies of the viruses provided by DSMZ (Germany, AS-0475, AS-0471). Samples were considered positive when OD_{405} value was at least twice that of the mean for the negative control.

Table 1. Disease ordinal scale for rating disease severity symptoms in bean and broad bean plants following inoculation with CMV and BYMV

Disease score	Disease symptom in bean	Disease symptom in broad bean
0	Symptomless	Symptomless
1	Mosaic	Very mild mosaic
2	Severe mosaic	Mild mosaic
3	Severe mosaic, Leaf blistering, Yellowing	Mosaic
4	Severe mosaic, Leaf blistering, Dwarfing, Yellowing	Severe mosaic

Disease severity was originally measured on an ordinal scale based on the severity of symptoms increasing from 0 (as symptomless) to 4 (as the most severe) and transformed to ratio scale (Disease Severity Index, DSI) in order to normalize the range from 0 to 1 using the following equation (Eq. 1) (Madden et al., 2007).

$$DSI = [(0 \times a) + (1 \times b) + (2 \times c) + (3 \times d) + (4 \times e)] / [(a + b + c + d + e) \times 4] \qquad (1)$$

where a, b, c, d and e are respectively the number of bean and broad bean plants infected with CMV and/or BYMV and scored from 0 to 4 according to their disease severities (Table 1). The equation normalizes the disease severity to proportions ranged from 0 to 1

interval by dividing the sum of products to the sum of the number of all plants scored multiplied by 4.

Area Under Disease Progress Curve (AUDPC)

AUDPC was used as an abstract variable that integrates the sum of disease measures over time. The AUDPC is calculated based on any two time points from distinct stages of the disease progress. For this purpose, AUDPC was approximated using trapezoid method by plotting a graph of disease severity against time and summing the trapezoids between time intervals (Shaner and Finney, 1977) by means of the following equation (Eq. 2);

$$AUDPC = \sum_{1}^{m-1} \left[\frac{y_i + y_{i+1}}{2} \right] [t_{i+1} - t_i] \qquad (2)$$

where n is the number of assessment times, y is disease measurement and t is time points (in days). Relative AUDPCs were calculated as proportion of maximum AUDPC by dividing the AUDPCs to 36 (Maximum AUDPC possible in 36 days).

Disease Response

For each viral and mock inoculated bean and broad bean plant, disease severity and plant growth parameters such as plant height and total fresh weight of aboveground tissues were evaluated and analyzed using SAS 9.1 (Anonymous, 1999). As the original scale for measuring disease severity was ordinal, to justify parametric ANOVA, assumption of homoscedasticity was investigated to examine whether disease scores in different groups had homogeneous variances. Additionally, disease severity data were also subjected to non-parametric ANOVA and Bonferroni posthoc tests to validate robustness of parametric tests when mild violations from parametric test assumptions were observed. Data on plant growth parameters were directly subjected to ANOVA and where significant main and interaction effects were found, Duncan's multiple range test was used to distinguish statistically significant differences among the means of treatments (Duncan, 1951). Factorial ANOVA was also performed to verify the virus interaction in either host species as statistical proof of the joint action.

To investigate the effect(s) of joint action of viruses on plant responses, Abbott's approach (Abbott, 1925) was also employed as a supplementary criterion. If the proportion of CMV (PCMV) and BYMV (PBYMV), respectively, was the plant responses due to exposure to the CMV and BYMV, 1-PCMV and 1-PBYMV were, respectively, the proportions of healthy tissues surviving infections when each virus was applied alone. If it was assumed that the two viruses affect different physiological targets in the host and act independently, infection to the mixture of viruses was estimated by the expected value of the proportion of healthy tissues survived given by the following formula (Eq. 3) (Kosman and Cohen, 1996):

PMix = Cexp= 1- (1-PCMV)(1-PBYMV)   (3)

where Cexp or PMix being the expected response to the application of the mixture. The effects of the two viruses and their mixture on the hosts were determined independently in an experiment and when the observed experimental effect of the mixture was equal to, higher or lower than their expected effect, additive, synergism, or antagonism interaction were declared, respectively.

RESULTS AND DISCUSSION

Disease Incidence and Severity

No significant differences were found in disease incidence between CMV and CMV+BYMV inoculated bean plants. However, disease incidence of BYMV inoculated bean plants was significantly lower than the incidence values recorded for CMV and CMV+BYMV inoculated bean plants. Significantly higher relative AUDPCs were recorded for CMV+BYMV followed by CMV infected bean plants while bean plants inoculated with BYMV showed significantly lower relative AUDPCs than the other two. No significant differences in relative AUDPC was observed in broad bean plants inoculated with CMV+BYMV, CMV and BYMV (Fig. 1). Final disease severity levels on proportional scale in bean and broad bean were recorded to be 0.80 and 0.38, respectively (Figs. 2 and 3). The slope of disease severity progress curve (DPC) was used as a measure of average rate of disease progress during the experiment.

In infected bean plants, the maximum slope was recorded for CMV+BYMV and CMV treatments, and BYMV infected bean plants showed the smallest rate of disease progress. BYMV DPC slope was significantly ($P < 0.01$) smaller than the slope values recorded for CMV+BYMV and CMV infected bean plants (Table 2). Although the rate of disease progress in bean plants infected with CMV alone and in mixed infection with BYMV (CMV+BYMV) was approximately the same, this value was 5.5-fold higher than that in plants infected with BYMV alone. Similar results were found for broad bean plants infected with the viruses, either alone or both viruses in mixed infection. In broad bean plants, CMV disease severity progress curve had the highest slope, but DPC slopes for CMV+BYMV mixed and BYMV infected broad bean plants were indistinguishable. In fact, DPC slope for CMV was 1.5-fold higher than the value of disease progress rate in CMV+BYMV mixed and BYMV infected broad bean but the differences between slopes in broad bean were not significant (Table 2). The mean time required to reach the final disease incidence levels from start was 10 and 16 days in broad bean and bean plants, respectively. After 16 dpi, all bean plants inoculated with CMV, BYMV and CMV+BYMV were infected, while in broad bean, only 40 percent of the inoculated plants showed viral infection. Progress of the disease severity in individual plants showed the same pattern in all pathosystems with a sharp increase in severity in the first two weeks followed by a slow progress through the

end of the fourth week (28 and 30 dpi for broad bean and bean, respectively) (Figs. 2 and 3).

Disease response based on Abbott's approach

Abbott's approach indicated synergistic main effect between CMV and BYMV on bean while the interaction was antagonistic when growth responses were considered on the same host. No synergistic or antagonistic interaction was found between the two viruses in broad bean. According to the Abbott's approach, the observed disease responses to co-infection of CMV and BYMV were greater than those expected in both bean and broad bean, and this suggests a synergistic effect if the interaction is evaluated based on the disease severity (i.e., Cobs >Cexp or synergy ratio>1). The symptom based synergy between the two viruses on bean plants verified by Abbott's approach was also supported by significant CMV*BYMV interaction based on analysis of variance and also significant differences between means of individual virus effects and their mixed effect. Comparison of the Abbott's expected growth responses (fresh weight and height) of CMV*BYMV with observed responses showed that the two viruses have antagonistic effects (i.e. Cobs <Cexp or synergy ratio<1). Antagonistic effect of CMV and BYMV on growth of bean could

also be verified by significant CMV*BYMV interaction effect ($P > 0.05$) and also mean comparison of growth responses (Table 3). Conforming to Abbott's approach, the two viruses affected disease severity on broad bean in an additive manner which was evidenced by synergy ratio near unity (Cobs ≈ Cexp). There were no significant differences between the mean of main individual virus effects and CMV*BYMV interaction; accordingly, the additive effect between the viruses could be declared in broad bean (Table 3). When Abbott's approach was applied to the growth responses (fresh weight and height), interactions were apparently additive (i.e. Cobs ≈ Cexp or synergy ratio ≈ 1) and the interaction between CMV and BYMV was non-significant ($P > 0.05$) and hence again additive effect was verified between the two viruses on broad bean (Table 3).

This type of interaction based on growth reduction was also supported by comparing means of CMV, BYMV and CMV+BYMV by Duncan multiple range test ($P< 0.05$) as the mean height and fresh weight of CMV+BYMV was not significantly different from those of CMV and/or BYMV (Table 3) and eventually only additive joint action between the two viruses in broad bean could be declared.
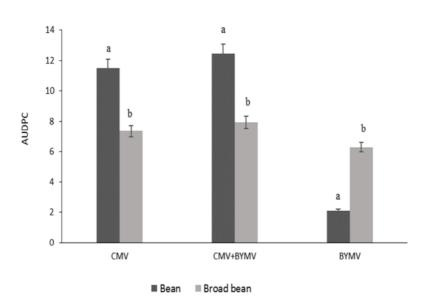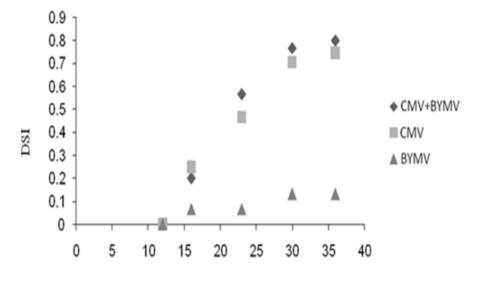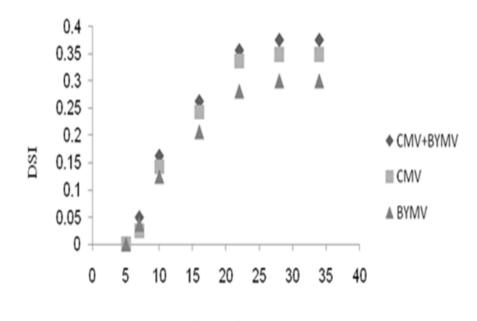

Fig. 1. Effect of single and double infection of CMV and BYMV on AUDPC in bean and broad bean. Bars with different letters are statistically ($P< 0.01$) significant.

Fig. 2. Effect of single and double infection of CMV and BYMV on disease severity in bean plants (cv. Bountiful)

Fig. 3. Effect of single and double infection of CMV and BYMV on disease severity in broad bean plants (cv. Lahijan)

Table 2. DSI slope* for curves of CMV, BYMV and their mixed infection on bean and broad bean plants

	Bean			Broad bean		
	CMV	BYMV	CMV+BYMV	CMV	BYMV	CMV+BYMV
DSI slope	0.021(a)	0.004(b)	0.022(a)	0.018(a)	0.012(a)	0.012(a)

* The values of DSI slope were calculated based on the following formula: DSI slope = $\Delta y / \Delta t$, where Δy is the difference between the maximum value of DSI and the minimum value and Δt is the difference between the maximum and minimum time in DSI curves ($P < 0.01$). The values are statistically significant at $P < 0.01$, when they share no common letter.

Table 3. Height and aboveground fresh weight of bean and broad bean plants not subjected to virus inoculation (control) or inoculated with CMV, BYMV, or a combined inoculum of CMV+BYMV at 40 dpi

Evaluation	Treatment	Broad bean			Bean		
		Fresh Weight	Height	Disease Severity	Fresh Weight (g)	Height (cm)	Disease Severity
A. DMRT mean comparison[1]	Mock	15.85a	12.66a	0.00b	13.26a	9.42a	0.00d
	BYMV	13.99a	12.24a	0.17a	9.77b	6.18b	0.06c
	CMV	14.49a	10.87a	0.20a	6.38c	4.85c	0.31b
	BYMV+CMV	13.46a	11.38a	0.22a	6.27c	4.03c	0.35a
B. P-value	BYMV main effect	*0.0001*	*0.1727*	*0.0002*	*<0.0001*	*<0.0001*	*<0.0001*
	CMV main effect	*0.0006*	*0.9182*	*<0.0001*	*0.0043*	*0.0002*	*<0.0001*
	CMV*BYMV interaction	*0.0628*	*0.0576*	*0.0014*	*0.0123*	*0.0551*	*0.0022*
C. Observed and Expected responses According to Abbott	Mock	100	100	100[2]	100	100	100
	BYMV	11.74	3.32	82.56	26.32	34.39	94.17
	CMV	8.58	14.14	79.61	51.89	48.51	68.92
	BYMV+CMV (C_{obs})	15.08	10.11	77.94	52.71	57.22	65.42
	Expected Response (C_{exp})	19.31	16.99	65.72	64.55	66.22	64.90
	Interaction[3]	ADD	ADD	ADD	ANT	ANT	SYN

[1] Duncan's multiple range test

[2] The percentage of healthy tissues survived infection in the columns labeled disease severity as response are 100% in mock, 100-DS% in virus inoculated plants, respectively.

[3] Joint action is concluded additive=ADD, synergistic=SYN and antagonistic = ANT based on three criteria: mean comparison (A), *P*-value of CMV*BYMV interaction (B) and expected response according to Abbott (C) (i.e. SYN if $C_{obs}>C_{exp}$, ADD if $C_{obs}≈C_{exp}$ & ANT

if $C_{obs}<C_{exp}$); (see the text for more details). The values are statistically significant at *P*<0.05, when they share no common letter.

Mixed viral infections are common in plants and may lead to a number of unpredictable interactions between the viral partners. The outcome of mixed infections may be additive, synergistic or antagonistic in nature which may have economic, epidemiological and biological significance (García-Cano et al., 2006; Hacker and Fowler, 2000; Malapi-Nelson et al., 2009; Martin and Elena, 2009; Sánchez-Navarro et al., 2006; Wintermantel et al., 2008).

The pattern of disease progress in singly and dually infected plants is also one of the efficient means in studying the joint action of plant viruses. The progress of disease severity in individual plants in all pathosystems tested in this study showed a sharp increase followed by a plateau. However, the rate of increasing disease severity in CMV and mixed infected bean plants were significantly higher. FDI=final disease incidence (& time to FDI) for bean and broad bean in response to CMV, BYMV and CMV+BYMV were 100% (16 dpi) and 38% (10 dpi), respectively. FDS=final disease severity (& time to FDS) for bean and broad bean in response to CMV, BYMV and CMV+BYMV were also >80% (30 dpi) and 35% (28 dpi), respectively.

When there are many disease measurements over time, the observations are summarized as AUDPC. It also facilitates comparisons between epidemics differing in the rate of disease progress and epidemic duration and may be normalized by dividing the AUDPC to the maximum AUDPC possible (Madden et al., 2007). In this study, relative AUDPC comparison among different treatments was in agreement with the results obtained when other measures of disease intensity were employed. The greatest relative AUDPC values in bean plants were recorded for CMV+BYMV followed by CMV and BYMV treatments (0.35, 0.31 and 0.06, respectively) while no significant differences were observed in the AUDPC values of mixed or singly infected broad bean plants (Fig. 1).

The assessment of disease severity based on synergy of CMV and BYMV on bean plants showed similar results to those of Murphy and Bowen (2006). They also showed that viral synergism in pepper caused by the mixed infection of CMV and Pepper mottle virus (PepMoV) could decrease various growth parameters including plant height, weight and yield, and may well be expressed by extremely severe symptoms. Also, in another study, growth rates of pepper plants were

greatly reduced in response to multiple infections of CMV, Pepper mild mottle virus (PMMoV) and PepMoV during the 30 to 40 dpi period under multiple-infection conditions. Simultaneous multiple infections of the three viruses were shown to induce more severe symptoms than did their single infections (Kim et al., 2010). Moreover, co-infection of CMV and BYMV reduced chlorophyll and carotenoids and also changed nutrient element values and virus accumulation (Tahmasebi et al., 2013).

Besides disease parameters, we also measured correlates of host plant fitness (i.e. plant growth characteristics) and evaluated the way they were affected by the viral solo/co-infections. Measuring plant fitness can be important in determining pathogen virulence. The effect of pathogen on plant growth has often been used to estimate virulence in plant-parasite interactions (Pagan et al., 2007). Such data are used in modeling the relationship between yield losses and disease severity and also for illustrating the tolerance of host plant to certain viruses. In this study, pathometric (disease intensity) and biometric (fresh weight and height) parameters of CMV, BYMV, and CMV+BYMV infected bean and broad bean plants were measured and compared as observed host responses against expected ones according to the Abbott's approach. Synergism is also defined as a joint action of mixture components where the combined effect is greater than the sum of the single component effects (Kosman and Cohen, 1996). In this study, relatively independent action of the two viruses through affecting different host physiological systems was assumed. This can imply that these two viruses employ different modes of action in their hosts. The effect of some factors such as the interacting viruses and their relative concentrations, plant host and its growth stage as well as the environmental conditions on the outcome of these interactions is unclear (Rentera-Canett et al., 2011). We employed three criteria to identify the interactions of the two viruses in the same host and a certain type of joint action was declared only if the three criteria were met. The first criterion (synergy ratio) was calculated as the ratio of observed response (Cobs) of the host plant infected by the two viruses to the expected responses (Cexp) of the same pathosystem according to Abbott's approach. An interaction was considered synergistic, additive or antagonistic if Cobs>Cexp, Cobs≈Cexp or Cobs<Cexp respectively. The second and third criteria were related to the statistical proof of the interaction to determine the statistical significance of the CMV*BYMV interaction (p<0.05). A significant difference was also investigated between the means of disease and/or growth response of the CMV+BYMV infected plants and singly infected ones. According to the Abbott method, in bean plants, the interaction of CMV and BYMV was synergistic when disease response was considered and antagonistic when the interaction was evaluated based on host growth responses. The amount of growth reduction in bean plants inoculated with CMV+BYMV did not cause a significant difference with those inoculated with CMV alone but was significantly different from bean plants infected with BYMV. In broad bean plants inoculated with CMV+BYMV, CMV and BYMV, disease severity, fresh weight and height were not significantly different from those of single virus infections although Abbott's approach recognized the type of viral interaction as synergistic when disease severity was used and antagonistic when growth reduction was considered. Accordingly, it was concluded that CMV and BYMV affect broad bean in an additive manner (Table 3).

Overall, broad bean may be recommended as a more appropriate host for growing in areas with high pressure of CMV and BYMV. According to our results, CMV has a predominant role in determining the outcome of the joint action of CMV and BYMV in bean plants; however, such specificity was not observed in broad bean. This fact in parallel with our findings clearly suggests a type of synergistic interaction between CMV and BYMV in mixed infected bean plants. Caution must be practiced in interpretation of such results as the interaction of the components is dose-dependent. Therefore, a reliable distinction between different types of interaction requires dose response curves of single and mixed components. Although it is common to utilize plant biomass as correlates of crop yield, development of yield loss models under field conditions is required to evaluate the impact of mixed infections on the yield of bean and broad bean. Results of this study are necessary to facilitate designing and evaluating improved strategies for successful disease management; however, further information on the effect of weather, host resistance, and also aphid transmission on yield losses in a given area is also required.

CONCLUSIONS

As the independent different action for the two viruses was assumed, Abbott approach was an appropriate method to investigate synergism, yet the outcome of the joint action is dependent on the type of parameter used for analysis, as the interaction was synergistic based on disease symptoms and antagonistic according to plant growth.

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روشی مناسب برای تعیین نوع برهمکنش ویروس موزائیک خیار و ویروس موزائیک زرد لوبیا

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چکیده- وقوع آلودگی همزمان پدیده‌ای رایج در گونه‌های گیاهی است که می‌تواند منجر به تغییرات در آلودگی ویروس‌ها شود. در این مطالعه، پیشرفت بیماری در آلودگی‌های مخلوط و انفرادی ویروس موزائیک خیار و ویروس موزائیک زرد لوبیا توسط اندازه‌گیری گسترش علائم، شدت بیماری و سطح زیر منحنی پیشرفت بیماری در لوبیا و باقلا بررسی شد. آلودگی همزمان لوبیا به ویروس موزائیک خیار و ویروس موزائیک زرد لوبیا، شدت بیماری بالاتر میزبان را باعث شد، اما تفاوت معنی داری در باقلا مشاهده نشد. روش Abbott اثر هم افزایی در لوبیا بین ویروس موزائیک خیار و ویروس موزائیک زرد لوبیا نشان داد، در حالی که بر پایه پاسخ‌های رشدی میزبان، برهمکنش از نوع آنتاگونیست بود. در باقلا، روش Abbott و پاسخ‌های رشدی میزبان، برهمکنش ویروس‌ها را از نوع افزایشی نشان داد. به طور کلی، روش Abbott روشی مناسب برای تعیین نوع برهمکنش از نوع هم افزایی در این پاتوسیستم‌ها بود.