

**Antioxidant responses of three Pepper
(*Capsicum annuum*) varieties against Pepper
Venal Mottle Virus**

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ABSTRACT

Aims: This study aimed to investigate the changes in antioxidant activity and protein content between non-infected and infected leaves of three *Capsicum annuum* varieties against Pepper Venal Mottle Virus.

Material and methods: PVMV isolated from infected pepper plants was inoculated to three healthy varieties of pepper (Pepper Narval, Yolo Wonder and Chili pepper) at an early stage of the growing period. Control peppers of each variety were treated in the same way with distilled water. The infection of inoculated plants was confirmed by the ELISA method. Control and infected leaves were collected three weeks after inoculation and used for biochemical analyses. Change in different biochemical parameters (catalase, superoxide dismutase, MDA and protein) in infected pepper plant was observed compared to control non-infected ones.

Results: CAT and SOD activities were increased in Pepper Narval and Pepper Yolo Wonder infected leaves compared to non-infected, while a significant decrease was observed in infected Chili pepper compared to control. Higher MDA content was found in Pepper Yolo Wonder and Chili pepper infected leaves ($P < 0.05$) than control while a non-significant difference was shown between the infected and non-infected of Pepper Narval variety ($P > 0.05$). Infected Chili pepper showed high protein content compared to control ($P < 0.05$). An opposite trend was observed in pepper Narval and Yolo Wonder varieties ($P < 0.05$).

Conclusion: The results of this study showed that PVMV infection induces changes in enzymes, malondialdehyde and protein levels. These biochemical components were greatly expressed differentially between PVMV infected and non-infected in Pepper Yolo Wonder variety. Further studies with more biochemical parameters may contribute to improve the pepper tolerance mechanism to PVMV in a breeding program.

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Keywords: Pepper; PVMV; catalase; superoxide dismutase; malondialdehyde; protein.

1. INTRODUCTION

Pepper (*Capsicum* spp.), including sweet pepper and hot pepper, is an important spice and vegetable crops worldwide [1]. Peppers belong to the Solanaceae family, genus *Capsicum* (Greenleaf, 1986). Among the five domesticated species of the genus *Capsicum* (*C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens*), *C. annuum* is the most widely grown in Africa (Grubben and El Tahir, 2004). In Burkina Faso, peppers are an important source of income for many small farmers. *C. annuum* crop is grown in open fields

24 on an estimated area of 1639 hectares with a production of 8230 tons/year [4]. However,
25 their average yields are severely affected by the presence of pests and diseases. Pepper
26 crop is infected by several fungal, bacterial, and viral diseases.

27 Among viral diseases, *Pepper vein mottle virus* is endemic and the most devastating
28 pepper virus and other solanaceous crops in several West African countries [5]. The virus
29 was first discovered in Ghana [6] and then in others West African countries [7,5], Ethiopia
30 [8], and South Africa [9].

31 *Pepper vein mottle virus* (PVMV) is a virus species in the genus Potyvirus of the family
32 Potyviridae [6]. PVMV is transmitted by aphids in a non-persistent manner and is
33 transmissible experimentally by mechanical inoculation. Symptoms expressed on the leaves
34 plants infected with PVMV are characterized by chlorotic vein banding, mottling, mosaic, and
35 distortion with puckering of leaves. Infected plants may show stunting with reduced and
36 distorted fruit set [10]. PVMV causes significant losses for growers of solanaceous crops in
37 several African countries [11, 12]. The incidence of the virus can reach 50 to 100%, leading
38 to significant losses of production causing whole field to be abandoned before harvest and in
39 some areas [13,14].

40 To control these pests, synthetic chemical pesticides are the most used solution. However,
41 several studies conducted in Burkina Faso [15,16] have highlighted the existence of poor
42 phytosanitary practices. The consequences are the intoxication of farmers and consumers,
43 environmental pollution and the selection of strains resistant to pesticides. Considerable
44 efforts have focused on the development of pepper varieties resistant to the virus. Early work
45 resulted in materials that were tolerant or only partially resistant [17,18].

46 Another approach to select resistant plant by using biochemical parameters was developed
47 [19,20].

48 Indeed, the contact of the plant with the pathogen induces biochemical reactions leading to
49 the production of defense substances. The level of antioxidant activity and total phenolic
50 content of peppers infected with the virus reflects the condition of resistance or susceptibility
51 of pepper plants [21]. The identification of pepper biochemical products expressed under
52 virus infection will be helpful to improve *Capsicum annum* tolerance mechanism to PVMV in
53 a breeding program. This study aimed to investigate the changes in antioxidant activity and
54 protein content of three peppers varieties against *Pepper Venal Mottle Virus* infection in
55 order to understand the biochemical tolerance mechanism of *Capsicum annum*.

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57 **2. MATERIAL AND METHODS**

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59 **2.1 Plant material and experimental dispositive**

60 Seeds of *C. annum* were purchased from a commercial supplier in Ouagadougou, Burkina
61 Faso. Three varieties of peppers, pepper Narval (Na), pepper Yolo Wonder (Y) and Chili
62 pepper were sown in pots (25 cm diameter) containing sterilized sand and peat (1:1). For
63 each variety, two seeds were sown in pots in three replications. A control group and infected
64 group were defined for each variety. Plants were well watered and grown in a greenhouse
65 under insect-proof conditions all the experiment.

66 **2.2 Inoculation of plants with *Pepper Venal Mottle Virus***

67 Virus isolates were obtained from the naturally PVMV infected pepper plants grown in
68 greenhouses and propagated in pepper plants. The isolated PVMV was confirmed
69 serologically by DAS-ELISA. The inoculum was prepared according to the method described
70 by Dikilitas et al. [22]. Each pepper variety was then inoculated with the supernatant
71 containing PVMV by gently rubbing on the leaves of 2-week-old seedlings [23]. Control
72 plants were treated in the same manner using distilled water. All tests were performed in
73 triplicate. After three weeks of inoculation, ELISA and biochemical tests were carried out.

74 **2.3 ELISA test**

75 Pepper leaf samples were tested for the presence of PVMV in inoculated peppers by double
76 antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Leaves were ground
77 in phosphate buffered saline with Tween 20 using a pre-chilled mortar and pestle. The
78 extract was tested using polyclonal antisera produced by SEDIAG. Samples were
79 considered positive when absorbance values at 405 nm (A405) were at least three times
80 greater than the mean absorbance value of five healthy control samples [24,11,25].

81 **2.4 Determination of antioxidant enzymes activities**

82 **2.4.1 Extraction of antioxidant enzymes**

83 500 mg of fresh leaves were homogenized with 50 mM sodium phosphate (pH 7.8) and
84 centrifuged at 4000 rpm during 10 minutes. The supernatant was used to measure
85 superoxide dismutase and catalase enzymes activities.

86 **2.4.2 Superoxide Dismutase (SOD) enzyme activity**

87 SOD activity was measured using the method described by Ranjitha and Vijiyalakshmi [26]
88 at 420 nm. The enzyme activity was expressed in terms of $\mu\text{mol/g}$ protein.

89 **2.4.3 Catalase (CAT) enzyme activity**

90 CAT activity was measured using the method described by Ranjitha and Vijiyalakshmi [26].
91 The absorbances were measured at 240 nm for each interval of 30 seconds during 3
92 minutes. The CAT activity was expressed in terms of μmol of H_2O_2 consumed/g protein.

93 **2.5 Lipid peroxidation assay**

94 The Malondialdehyde (MDA) content as the marker of lipid peroxidation was determined as
95 described by Mahi et al. [27]. The MDA content of samples was expressed in micromole per
96 milligram ($\mu\text{mol.mg}^{-1}$) of leaves fresh weight.

97 **2.6 Protein content**

98 500 mg of leaves were homogenized in 5 ml of 0.1 M NaCl. The samples were centrifuged at
99 4400 rpm during 30 min, and the supernatant was used to determine the protein content.
100 Protein concentration was determined by Bradford method as described by Mimouni et al.
101 [28].

102 **2.7 Statistical analysis**

103 The results are presented as mean \pm SD for triplicate analysis and were subjected to one-
104 way analysis of ANOVA variation with Tukey's Significant Difference test and $p < 0.05$ was
105 considered significant. The statistical analysis was performed using XLSTAT Version Pro-
106 2017 and the graphs were drawn using Graph Pad Prism software version 5.0.

107 **3. RESULTS**

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109 **3.1 Pathogenicity test**

110 The different varieties of pepper inoculated by PVMV showed more or less severe
111 symptoms. Inoculated Chili pepper developed disease symptoms 2 weeks after inoculation
112 and developed severe symptoms such as chlorotic vein banding, mottling, mosaic, and

113 distortion. However, the inoculated Pepper Na and Pepper Y varieties developed slight
114 symptoms of chlorotic vein banding on some leaves three weeks after inoculation. These
115 observations were confirmed by ELISA-positive result for PVMV. On the contrary, no
116 symptoms were observed in control peppers plant and confirmed by ELISA-negative result
117 for PVMV. The results of the pathogenicity test are presented in Fig. 1.

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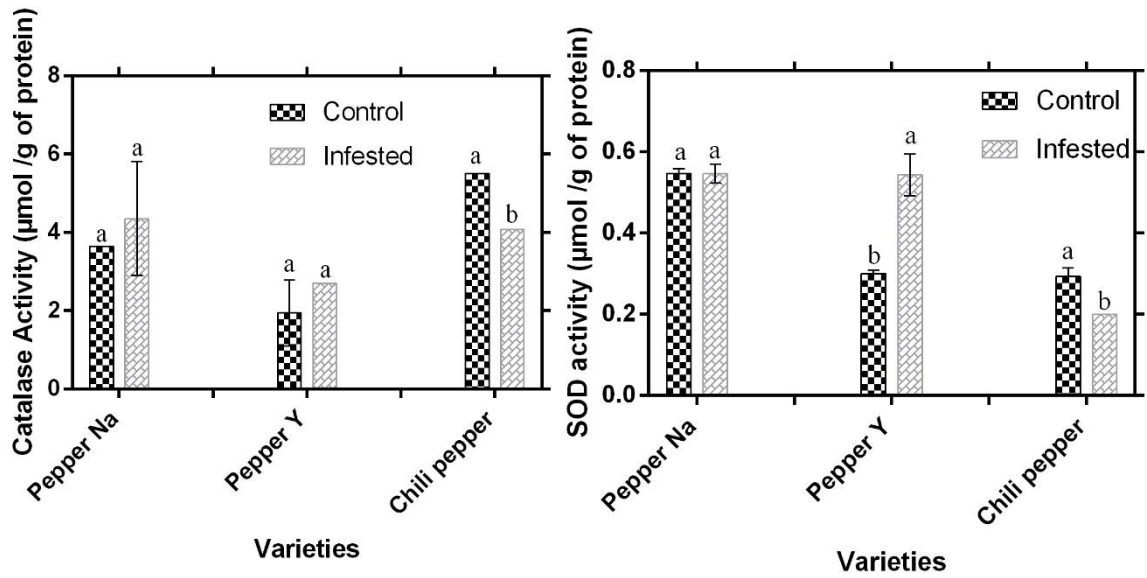
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Fig. 1. Pepper leaf structures (a) healthy, (b) infected

121 **3.2 Enzymes antioxidant activities of non-infected and infected pepper** 122 **varieties**

123 Catalase (CAT) and superoxide dismutase (SOD) activities of control and PVMV-infected
124 peppers are shown in Fig. 2. The activities of these enzymes were increased non-
125 significantly in infected pepper Na variety compared to control ones. The activity of catalase
126 enzyme increased non-significantly while the SOD enzyme activity increased significantly (P
127 < 0.05) in infected pepper Y as compared to non-infected. A significant decrease ($P < 0.05$)
128 in catalase and superoxide activities was observed in Chili pepper variety.

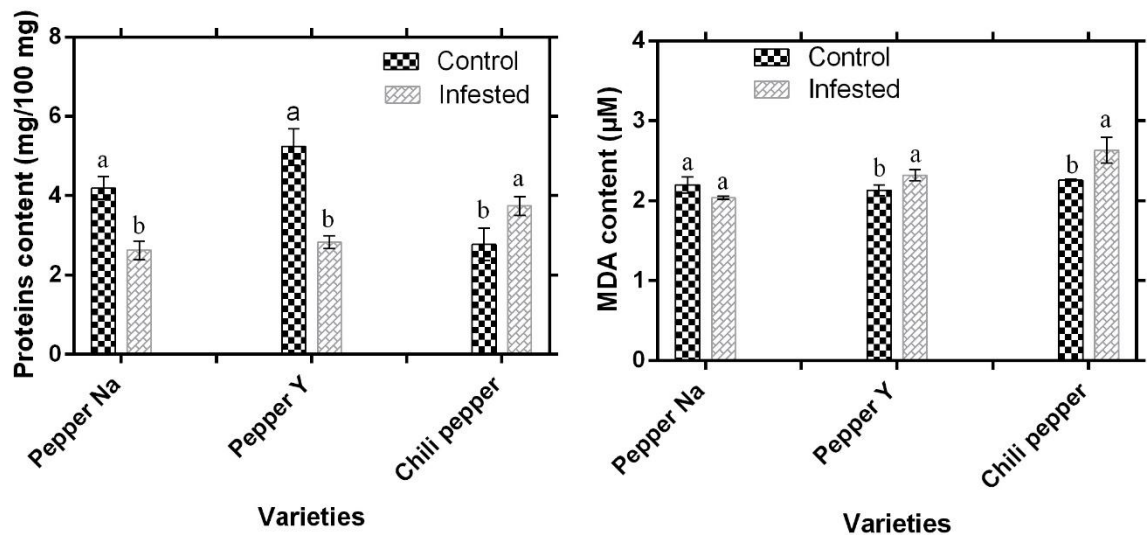


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130 **Fig. 2. Enzymes antioxidant activities of non-infected and PVMV-infected varieties of**
 131 **pepper**

132 **3.3 Lipid peroxidation of non-infected and infested pepper varieties**

133 The MalonDiAldehyde (MDA) content of control and PVMV-infected peppers is shown in Fig.
 134 3. The results showed non-significant decrease of MDA content only in infested Pepper Na
 135 variety compared with control ones. On the contrary, the MDA content of Pepper Y and Chili
 136 pepper varieties infested with PVMV significantly increased ($P < 0.05$) when compared to
 137 control ones.



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139 **Fig. 3. Protein and MDA contents of non-infected and PVMV-infected varieties of**
140 **pepper**

141 **3.4 Proteins content of non-infected and infected pepper varieties**

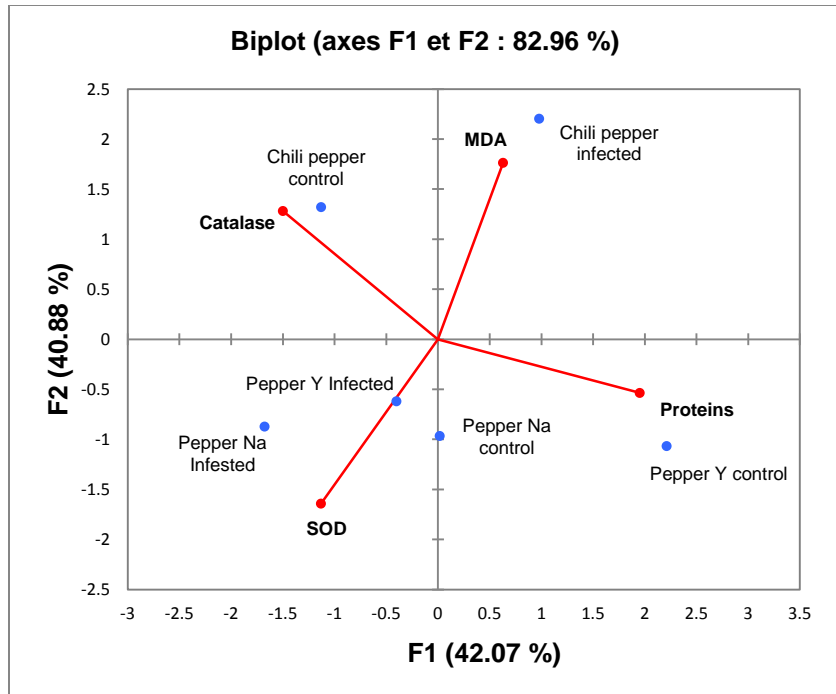
142 The protein content of control and PVMV-infected peppers is shown in Fig. 3. The protein
143 content differed significantly ($P < 0.05$) among control and infected of the three varieties of
144 pepper. The results revealed that the protein content decreased in infected pepper Na and
145 Y. However, Chili pepper showed significantly increased in protein content due to PVMV
146 infection compared with healthy ones.
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148 **3.5 Comparative analysis of the protein content and antioxidant response of** 149 **pepper varieties to PVMV infection**

150 The principal component analysis was performed on the basis of variations in enzyme
151 antioxidant, MDA and proteins contents of different pepper varieties three (3) weeks after
152 inoculation. Fig. 4 presents the repartition of different parameters evaluated in the biplot axis.

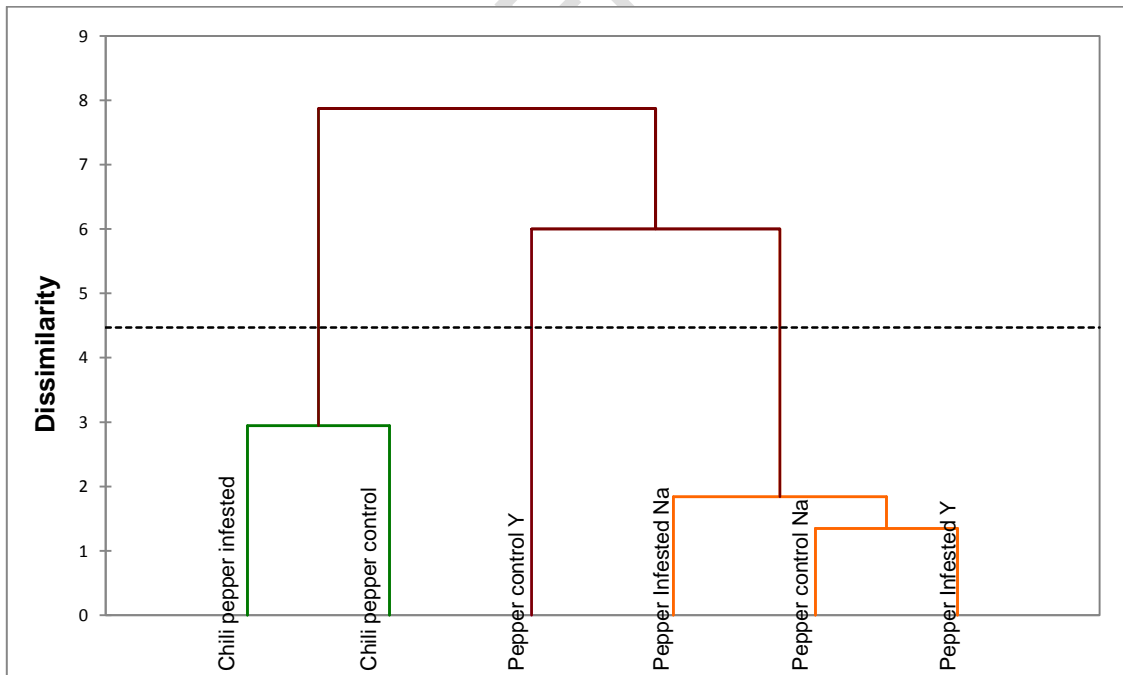
153 The first two principal components explained 82.96 % of the total variance. The first principal
154 component (F1) and the second principal component (F2) account, respectively for 42.07 %
155 and 40.88 % of the total variation. The first principal component (F1) separated Chili pepper
156 control from Chili pepper infected in one hand and Pepper Y infected, Pepper Na infected
157 from Pepper Y control and Pepper Na control in other hand. The F1 axis divided the control
158 non-infected group from infected group of the different varieties of pepper. The second
159 principal component (F2) separated Chili pepper (control and infected) from Pepper Y and
160 Pepper Na (control and infected). The F2 axis divided the pepper in different part according
161 to the type of pepper variety. Analysis of the correlations between the evaluated variables
162 and factors showed a strong contribution of Pepper Y infected, Pepper Na infected and
163 Pepper Na control to SOD while Chili pepper infected contributes strongly to MDA. Pepper Y
164 control contributes strongly to proteins, while Chili pepper control contributes strongly to
165 catalase. After the construction of dendrogram of the different treatments, the treatments
166 were grouped into three main classes, I, II and III (Fig. 5). Class I comprised Pepper Na
167 control, Pepper Na infected and Pepper Y infested. Class II comprised Chili pepper control
168 and Chili pepper infected. Class III is constituted only of Pepper Y control.

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171 **Fig. 4. Principal component analysis of the different pepper varieties responses on**
 172 **the protein content and antioxidant activities to PVMV infection**



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174 **Fig. 5. Dendrogram of the different pepper varieties response based on the protein**
 175 **content and antioxidant activities to PVMV infection.**

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177 **4. DISCUSSION**

178 The results of the pathogenicity test showed an early development of diseases symptoms
179 and severity in the Chili pepper variety due to PVMV infection compared to the Pepper Na
180 and Pepper Y varieties. Besides, the ELISA tests were positive for all infested peppers. The
181 Chili pepper showed to be more sensible to PVMV infection compared to the others varieties
182 of pepper. The susceptibility of Chili pepper is confirmed by the decrease of catalase and
183 superoxide dismutase activities after PVMV infection when compared to non-infected group.
184 Only pepper Y variety showed a significant increase of SOD activity in infected group
185 compared of non- infected group. The pepper Yolo Wonder variety is tolerant to PVMV by
186 increasing the activity of superoxide dismutase, enzyme involved against the biotic or abiotic
187 stress of plant. Appiah et al. [29] showed that pepper plants respond differently to a viral
188 infection based on their susceptibility or resistance to viruses. Antioxidant enzymes (CAT,
189 SOD) are produced by host plant to promote cells protection of oxidative damage from
190 pathogens [30]. They induce resistance against pathogen [31]. Similar results have been
191 reported by Siddique et al. [32]. They showed an increase of the CAT and SOD activities in
192 the leaves of resistant varieties of cotton and a decrease in the susceptible varieties after
193 infection with *Cotton Leaf Curl Burewala Virus*.

194 Generally, infected plants show a high content of protein, which could be due to both of the
195 activation of the host defense mechanism and the pathogen attack mechanism [33]. In this
196 study, protein content significantly decreased in infected Pepper Na and Pepper Y compared
197 to control non-infected. An opposite trend was observed in Chili pepper plants. The increase
198 in protein content in Chili pepper after infection may be due to viral replication which could
199 explain it high susceptibility to PVMV. Indeed, Zinga et al. [34] showed that protein content is
200 higher in cassava leaves infected by *African Mosaic Virus* than in healthy ones. However,
201 other investigators have shown an increase in protein content in resistant infected varieties
202 [32,35].

203 MDA is a general indicator of lipid peroxidation [36]. MDA produced during lipid peroxidation
204 is an indicator of cellular membrane damage to the cell membrane caused by pathogenic
205 infection [27]. Infection of Pepper Y and Chili pepper with PVMV resulted of an increase of
206 the MDA content compared to control non-infected. Previous studies have shown that MDA
207 content tends to increase in susceptible varieties due to infection. Lanubile et al. [37]
208 obtained the same result with maize leaves corn infested by *Aspergillus niger*. Analysis of
209 the principal components revealed a negative correlation between MDA and SOD. Chili
210 pepper infected contributes strongly to MDA while infected Pepper Na and infected pepper Y
211 contribute strongly to SOD. MDA increasing translates cellular degradation while SOD
212 enhancement induces cellular defense mechanism [27,31]. The Pepper Na and Y varieties
213 produce chemicals inducing resistance to PVMV than Chili pepper. Sama et al. [35] showed
214 that the leaves of susceptible varieties of *Jatropha* strongly contribute to the MDA content
215 after infection with *Lasiodiplodia theobromae*.

216 Combination in classes of three varieties of uninfected and infected peppers revealed a
217 relationship between control and infected Chili pepper in class II. This closeness might be
218 due to a weak response of the measured parameters (protein and MDA content, antioxidant
219 enzymes activities) of this variety to the viral infection. Chili pepper presented a susceptible
220 reaction against the PVMV. Pepper Y control and Pepper Y infected are in different classes.
221 Likewise, Pepper Na control and infected are in the same class but in different subclasses.
222 This may explain by the important biochemical response of Pepper Na and Y varieties due to
223 viral infection.

224 In view of parameters of the oxidative enzymes (CAT, SOD) and MDA of the infected pepper
225 varieties then the grouping into classes of the different uninfected and infected varieties, we

226 can conclude that Pepper Y induce resistance against PVMV infection than Pepper Na and
227 Chili pepper.

228 **4. CONCLUSION**

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230 This study found variations in the activity of oxidative enzymes, MDA and protein contents
231 between the three infested and non-infested pepper varieties. SOD is involved in the
232 biochemical defense mechanisms controlling the development of PVMV in Pepper Yolo
233 Wonder variety. Under stressful conditions such as viral infection, stimulation of biochemical
234 parameters plays a vital role in the defense mechanism. The results of this study suggest
235 that the Pepper Yolo Wonder variety is more tolerant to *Pepper Veinal Mottle Virus* than the
236 Pepper Narval and Chili pepper varieties. Further studies with more biochemical parameters
237 related to pathogenicity may contribute to improve the pepper tolerance mechanism to
238 PVMV in a breeding program.

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241 **COMPETING INTERESTS**

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243 Authors have declared that no competing interests exist.

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UNDER PEER REVIEW