# Inhibitory effects of the extract from *Quercus dentata* gallnut against plant virus infection

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Key words : CMV, PMMoV, Daimyo Oak gallnut, Inhibitor,

## Abstract

*Pepper mild mosaic virus (PMMoV) and cucumber mosaic virus (CMV) are important pathogens in various vegetable crops worldwide. We have found that methanol extracts of Quercus dentate (Daimyo Oak) gallnut strongly inhibit PMMoV and CMV infection. Based on this result, the inhibitor named as "KN0912" formulated from the extract of Q. dentate gallnut was tested for its inhibitory effects on PMMoV or CMV infection to each local lesion host plant (Nicotiana glutinosa; PMMoV, Chenopodium amaranticolor; CMV). Pre-treatment effect of KN0912 against infections of each virus to local host plant was measured to be 75.1±0.5~97.5±1.5% to PMMoV and 70.6±2.2~99.0±1.0% to CMV in 1~10mg/ml conc. and the absorption effect of the antiviral composition of KN0912 to the inside of tobacco leaves tissue, was inhibited by 55.7% to PMMoV and 63.8% to CMV. The persistence of KN0912 treatment was maintained until after the 3 days high inhibitory effect by 98% to PMMoV and by 95.1% to CMV. Inhibitory effects on systemic host plants of KN0912 were measured to be 80~90% to PMMoV and 60~75% to CMV. From the change of morphological characteristics of PMMoV particles under EM, we are tentatively suggested that one mode of action of KN0912 is inactivation due to the destruction of virus particles.*

## Introduction

*Pepper mild mottle virus* (PMMoV), a genus *Tobamovirus*, is one of the most important pathogens of pepper. Also, *Cucumber mosaic virus* (CMV), a genus *Cucumovirus*, has a worldwide distribution and a very wide host range. This study was undertaken to develop of environmental-friendly antiviral agent using natural materials of plant resources. Several substances have been reported as plant viral inhibitors, such as milk, polysaccharides (Sano,1999). Many plant resources have been reported to have potent antiviral activity and some of them have already been used to treat animals and people who suffer from viral infection (Hudson, 1990), because they virtually constitute a rich source of bioactive. However, little work has been done to control plant viruses by using these natural products in spite of their excellent pharmacological signification. In this study, we found the ethanol extracts from gallnut of Daimyo oak tree which strongly inhibited the infection of PMMoV and CMV. Here we report several properties of the antiviral activities by KN0912 formulated from the extract of *Q. dentate* gallnut.

## Materials and methods

**Prepared samples and extracts.** The fresh Gallnut of *Q. dentata* was sampled at Gangnung in Korea and the voucher specimen was deposited and maintained at the Herbarium of SPES, Korea. The dried sample(1kg) was ground using a blender and

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extracted twice with ethanol(7L) at room temperature and filtered. The filtrate was concentrated *in vacuo* at 40℃ and freeze-dried.

**Antiviral activity of KN0912.** *Pepper mild mottle virus* (PMMoV) and *Cucumber mosaic virus* (CMV) were used as virus sources. *Nicotiana glutinosa* was used for local lesion assay of PMMoV infection, while *N. tabacum* cv. Samsun was used for systemic infection in the greenhouse. For the virus inoculums, tobacco leaves (0.1g, cv. Samsun) systemically infected with PMMoV, were grounded in 20ml of phosphate buffer (10mM, pH 7.2), filtered and used as PMMoV inoculation. The inoculums of CMV was prepared from *N. tabacum* cv. Samsun NN with similar method mentioned above. *Chenopodium amaranticolor* was used for local lesion assay of CMV infection, while *N. tabacum* cv Samsun NN was used for systemic infection. Antiviral activity in local lesion host was tested by using the half-leaf method. For the antiviral activity in the systemic host, the KN0912 was sprayed onto the entire surface of the systemic hosts, cv. Samsun and cv. Samsun NN. Viruses were inoculated mechanically.

**Observation of electron microscopy.** 1% KN0912 was mixed with an equal volume of 100ul/ml PMMoV solutions in 10mM phos. buffer. The samples were examined with a TEM electron microscope.

## Results

**Inhibitory activity of KN0912.** The ethanol extract from gallnut of *Q. dentata* was tested against PMMoV by local lesion assay on *N. glutinosa*. Based on these result, the inhibitor named as "KN0912" formulated from the ethanol extract of *Q. dentate* gallnut was tested for its inhibitory effects on PMMoV and CMV infection to each local lesion host plant. First of all, the mixture treatment effect of KN0912 against infections of each virus (PMMoV and CMV) to local infection plant was measured to be 100% to PMMoV and 100% to CMV in 10mg/ml conc. Also, as shown in Table 1, pre-treatment effects of KN0912 against infections of each virus to local host plants were highly estimated to be 75.1±0.5~97.5±1.5% to PMMoV and 70.6±2.2~99.0±1.0% to CMV in 1~10mg/ml conc. These effects were better than ones of the known viral inhibitors such as Lentemin (Oka *et al.* 2008) 10mg/ml of which reduced local lesions to approximately 90% to compare of the control.

Tab. 1: Pre-treatment effect of KN0912 against infections of *Pepper mild mosaic virus* (PMMoV) or *Cucumber mosaic virus* (CMV)

| Treatment | Concentration (mg $mL^{-1}$) | Inhibition (%)** | |
|---|---|---|---|
| | | PMMoV | CMV |
| KN0912* | 10 | 97.5±1.5*** | 99.0±1.0 |
| | 5 | 93.0±1.2 | 93.3±0.6 |
| | 2 | 80.2±2.4 | 84.0±0.5 |
| | 1 | 75.1±0.5 | 70.6±2.2 |
| Water(control) | - | 0.0±0.0 | 0.0±0.0 |

* Diluted KN0912 was treated to 2 hr prior to mechanical inoculation of each virus to host plant

** Inhibition % = (1- No. of local lesions on tretment/No. of lesions on control) x 100.

*** Each value represents the mean±standard deviation of three replicates.

In order to assay the absorption of the antiviral composition of KN0912 to the inside of the leaf tissue, the extract(10mg/ml) were applied on the backside of the half leaves of host plants (*N. glutinosa* or *C. amaranticolor*), viruses infection onto the upper surface were inhibited by 55.7% to PMMoV and 63.8% to CMV. (Fig 2). These results

indicated that the inhibitory effects of KN0912 were induced not only by barrier effects, but also by some other unclear antiviral effects.

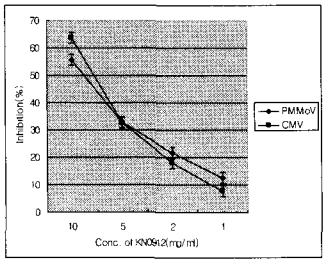


Figure 1: Absorption effect of KN0912 to the inside of the leaf tissue. Dilutions of KN0912 were applied on the backside of half leaf of host and the viruses (PMMoV or CMV) were inoculated on the upside of leaves, respectively. Each point represents the mean of three trials and the vertical bars indicate SE ranges.

**Effects of the time of treatment.** In order to assay the persistence of KN0912 treatment, the extract (10mg/ml) was applied on the leaves of host plants (*N. glutinosa* or *C. amaranticolor*), The KN0912 showed a higher inhibitory effect as 98% to PMMoV and as 95.1% to CMV until after the 3 days, but the effect of inhibitory was significantly reduced up to 25% at 5 days (Fig 2).

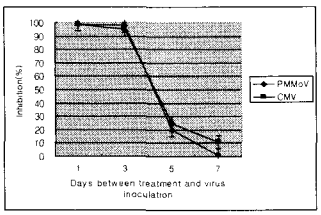


Figure 2 : Duration of inhibitory activity of KN0912 against PMMoV and CMV infection on host plants. Each point represents the mean of three trials and the vertical bars indicate SE ranges.

**Antiviral effects in the systemic host.** When the KN0912 was sprayed 2 hours before virus inoculation, PMMoV or CMV infections through the leaves of their systemic host were remarkably reduced in greenhouse condition. This result showed that the inhibitory activity of KN0912 was superior to the effects of the known viral inhibitors such as skim-milk or Lentemin (Oka et al. 2008). The KN0912 used for this study was apparently harmless to the tobacco seedlings. Judging from the fact that there's no change of leaf colours and there's no symptoms of growth inhibition.

Tab. 3: Systemic inhibitory effects of KN0912 against PMMoV or CMV infection on the host plants, respectively

| Treatment * | No. plants infected / inoculated ** | | | |
|---|---|---|---|---|
| | PMMoV | | CMV | |
| | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| KN0912 | 2/20 | 4/20 | 12/20 | 15/20 |
| Skim milk | 7/20 | 12/20 | 9/20 | 5/20 |
| Water(control) | 20/20 | 20/20 | 20/20 | 20/20 |

* Experiments were repeated twice with 20 replicate seedlings for each treatment of KN0912 (conc. 4mg/ml) and Skim milk (conc. 100mg/ml).

** Five~six week old tobacco seedlings (cv. Samsun and Samsun NN) were sprayed with treatment indicated (200ml/20pots), and each inoculums was rubbed onto 2 leaves of each plant 2 hrs after treatment. Inhibition was confirmed 4 weeks after inoculation by ELISA

**Electron microscopy of PMMoV in the absence or presence of KN0912.** The PMMoV particles were almost destroyed or segmented by mixing KN0912, but not affected in the absence of KN0912. Therefore, it is thought that one mode of action of KN0912 is inactivation of the virus due to the destruction of PMMoV particles .

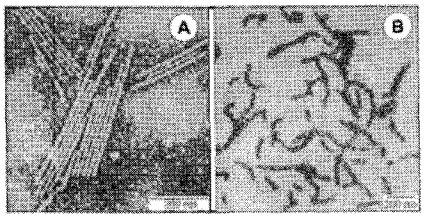


Figure 3 : Electron micrographs of PMMoV particles in the absence(A) or presence of KN0912 (B), Bar=200 nm.

## Discussion

This report presents the first evaluation of antiviral activities of *Q. dentata* (Daimyo Oak) gallnut to plant viruses. The gallnut is a plant excretion produced when irritants are released by the larvae of gall insects. It contains high amounts of tannic acids such as gallic acid and ellagic acids. As the gallnut extract is widely used in pharmaceuticals, food and feed additives and dyes, it is safe natural material which can be used in organic agriculture. The gallnut extract used in this report also proved harmless to tobacco seedlings. Our results indicate that it is a potent virus inhibitor that may be used to prevent the spread of plant virus infections in the field. Previously, we reported that the extracts of *P. linteus* and *G. rhois* were sources of potent inhibitors against several plant virus infections (Kwon *et al.*, 2010). The mechanisms that these natural materials inhibit PMMoV and CMV infection have not been completely elucidated thus far. But from the change of morphological characteristics of PMMoV, we tentatively conclude that one mode of action of KN0912 is inactivation due to the segmentation of virus particles. Future work will be done and discussed more in detail the antiviral components and its mechanism of *Q. dentata* gallnut.

## Conclusions

The inhibitor named as KN0912 formulated from the *Q. dentata* (Daimyo Oak) gallnut extract strongly inhibit PMMoV and CMV infection. Our results indicate that KN0912 is a potent virus inhibitor that may be used to prevent the spread of plant virus infections in the field.

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