The introduction of the new control method against plant viruses infection for organic farming

S.B. Kwon and J.S. Chung

Gangwon Agricultural Research and Extention Service, Korea
BIG Co., Ltd. Korea

1. INTRODUCTIONS

1. **Plant virus disease**
   - represent significant threats to modern agriculture
   - obligate intracellular parasites
   - do not have the molecular organelle to replicate without a host plant

2. **Control of plant viruses**
   - Crop rotation
   - Removal or avoidance of source of infection in or near crops
   - Virus-free seeds and vegetative stocks
   - Control or avoidance of vectors
   - Cross-protection
   - Breeding for virus resistance plants
   - Antiviral agents

2. Purpose

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

However, recently little work has been done to control plant viruses by using these natural products in spite of their excellent pharmacological signification.

This study was undertaken to develop of environmental-friendly antiviral agent using natural materials of plant resources.

Main ingredient of Kimchi
Cabbage, **Pepper**, garlic etc...

Kimchi

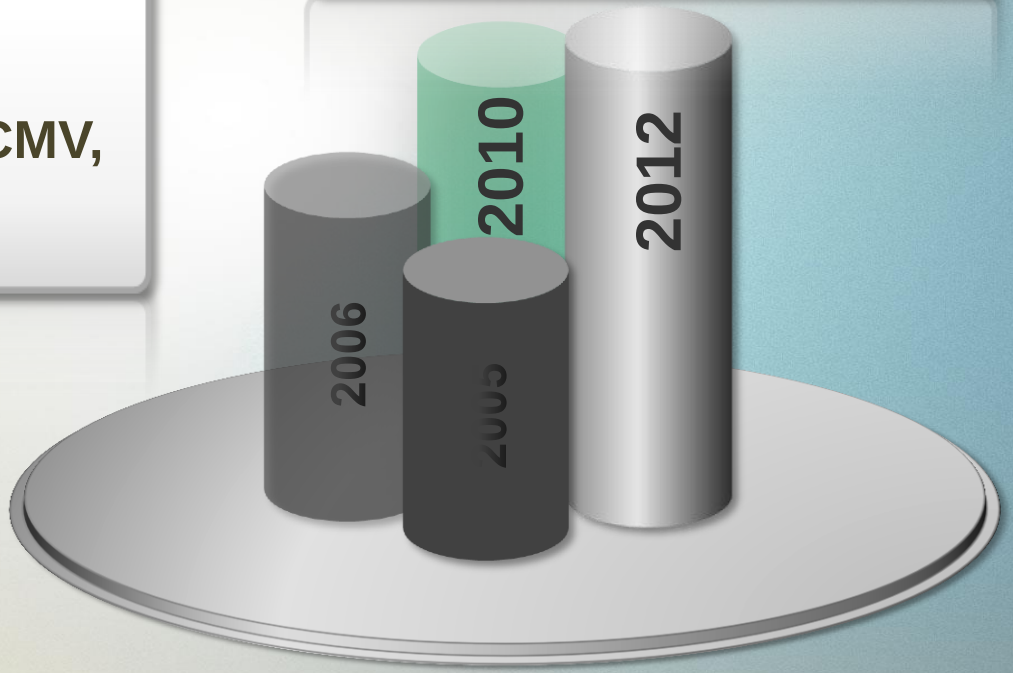
Plantation dimension of
Pepper in Korea
45,000 ha

2010, 2012

30- 40% : PMMoV, CMV, PepMoV, PVY, TSWV

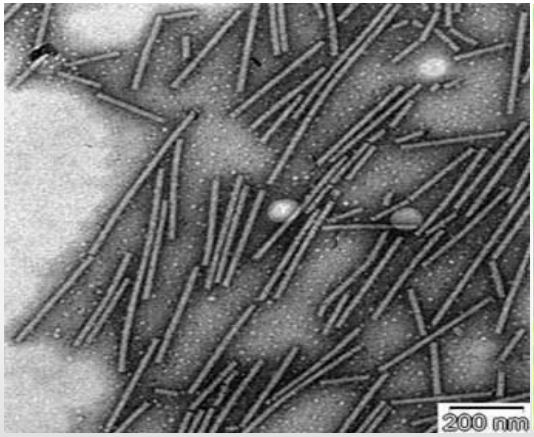
2005, 2006

10-20% : PMMoV, CMV, PepMoV



The incidence of pepper viruses in Korea

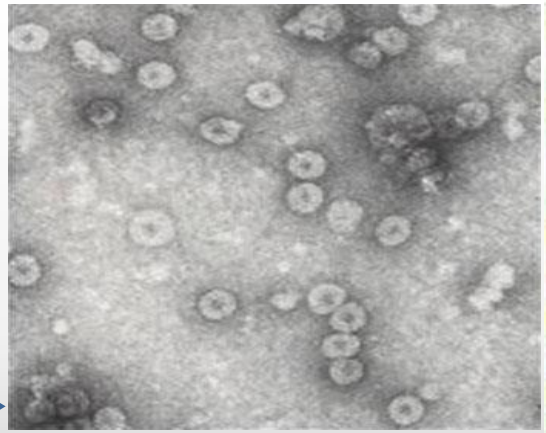
pepper mild mottle virus (PMMoV)



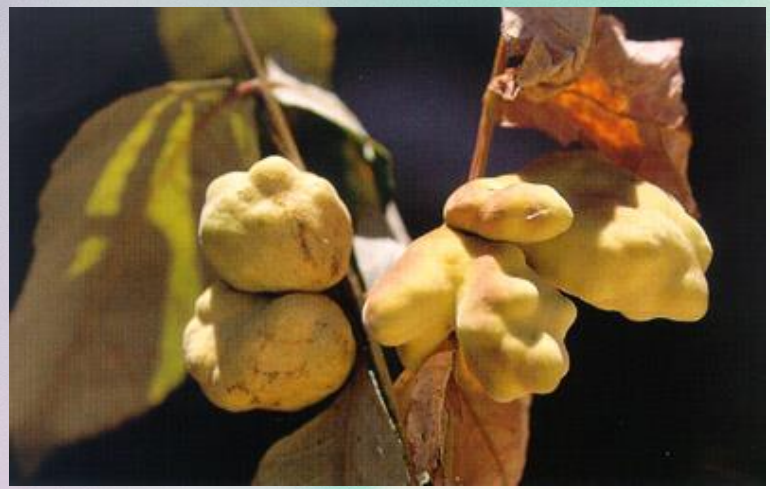
- Genus Tobamovirus
- Size: 310x18 nm
- Transmitted by sap and soil etc.
- Symptom: mosaic.

cucumber mosaic virus (CMV)

- Family Bromoviridae, genus cucumovirus
- Round particle of Diameter 30nm.
- Transmitted by aphids and sap etc.
- Yellow mosaic.



3. Materials and Methods



Gall of *Rhus javanica*

Gall of *Quercus dentata*

Qbyrus-1

4. Antiviral activity assay

Test 1.
Local lesion infection test

Used virus

Pepper mild mottle virus (PMMoV), *Cucumber mosaic virus* (CMV)

Used plant

PMMoV :
N. glutinosa
CMV :
C. amaranticolor

Assays

Mixed treatment
Pre-treatment
Leaf backside tests

Treatments

Conventional half-leaf method

Inhibitory ratio(%)

(Untreated –Treated) /Untreated x100

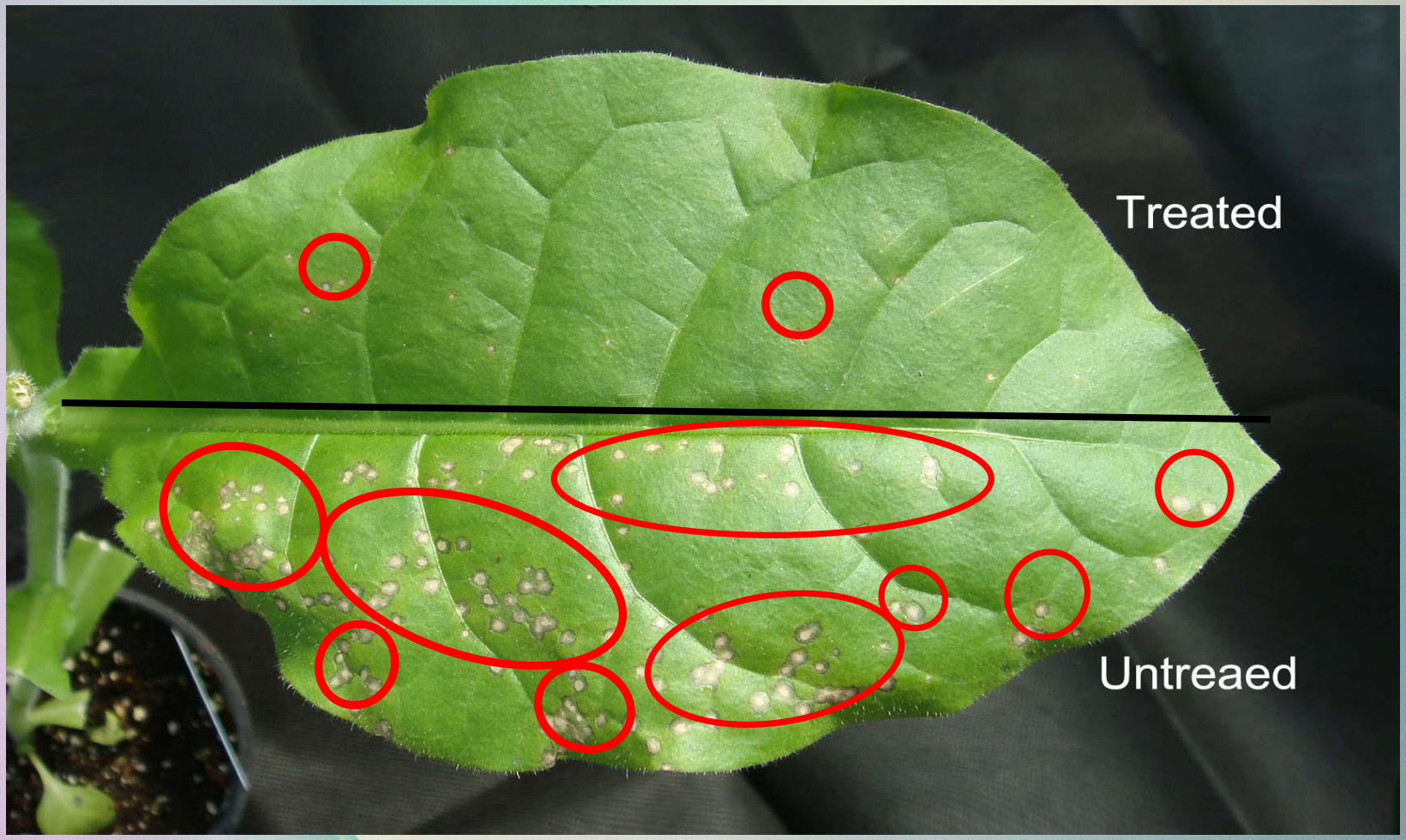


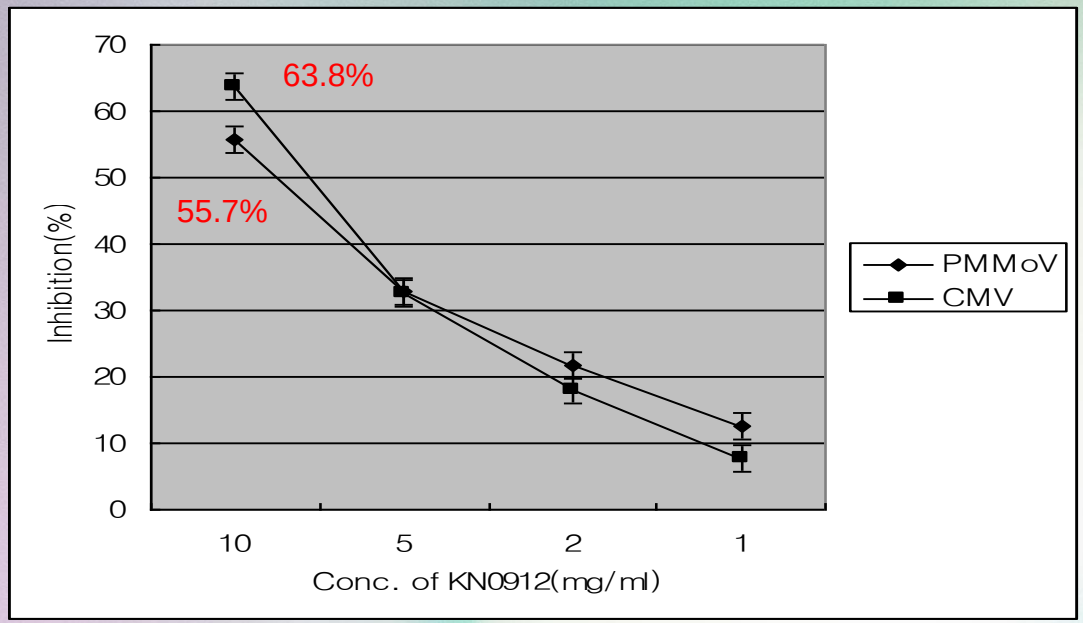
Fig. Inhibitory effect of local lesion symptom induced by Qbyrus-1

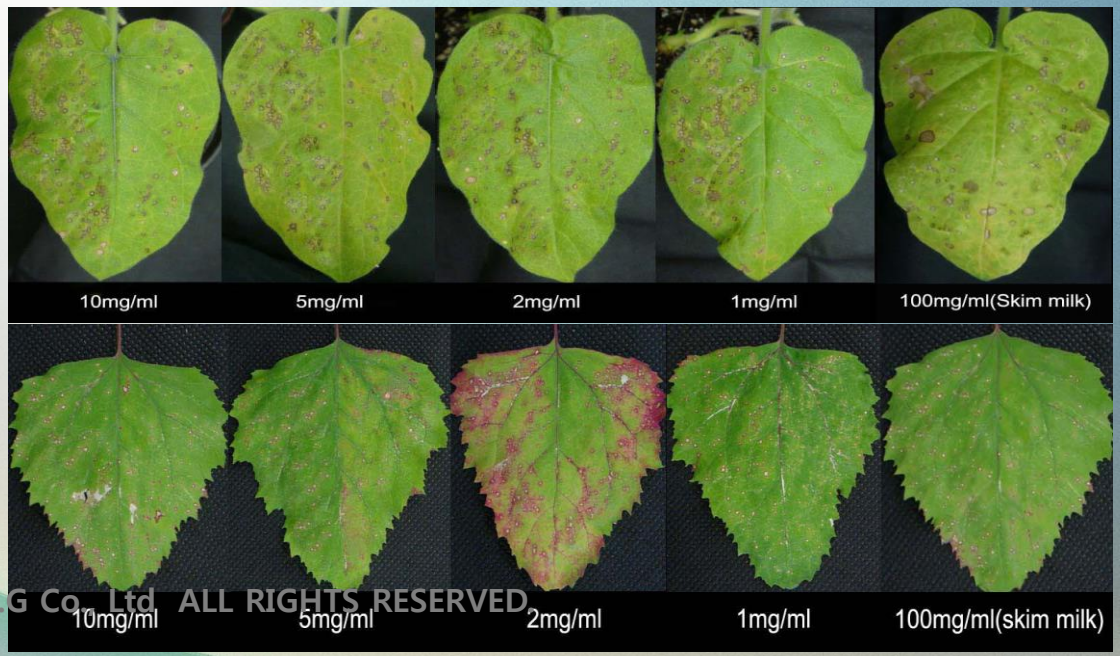
Table. Inhibitory effects of Qbyrus-1 against local infection of PMMoV and CMV

Treatment	Concentration (mg mL^{-1})	Inhibition ratio(%)	
		PMMoV	CMV
Qbyrus-1	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

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.

Fig. Absorption effect of Qbyrus-1 to the inside of the leaf tissue





Test 2.
Systemic infection test

Used virus

Pepper mild mottle virus (PMMoV),
Cucumber mosaic virus (CMV)

Used plant

PMMoV : *Tobacco* (cv. Samsun)

CMV : *Tobacco* (cv. Samsun NN)

Method

Spraying to foliage treatment

Treatments

Conc. : 4mg/ml
Tested plant : 20 pots

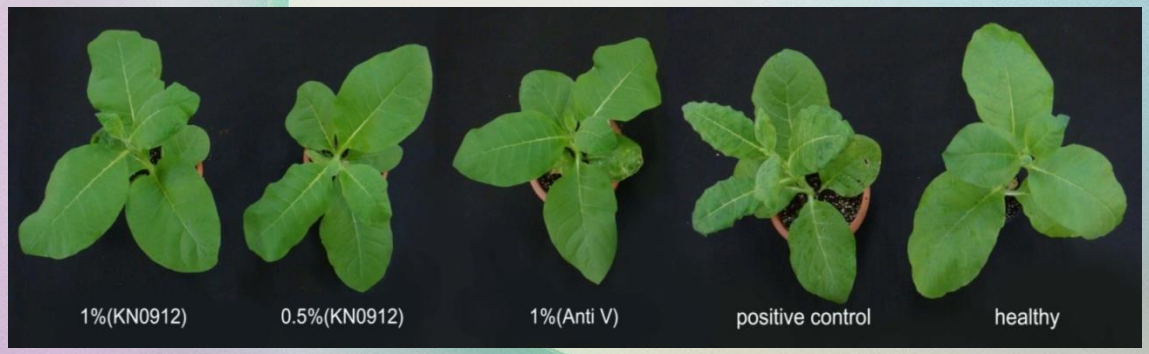
Result

Confirmed 4 weeks after inoculation by ELISA or symptoms

Systemic infection – PMMoV & CMV

Table . Inhibitory effects of Qbyrus-1 against systemic infection of PMMoV or CMV

Treatment *	No. plants infected / inoculated **			
	PMMoV		CMV	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Qbyrus-1	2/20	4/20	8/20	5/20
Skim milk	7/20	12/20	9/20	10/20
Water(control)	20/20	20/20	20/20	20/20



PMMoV / Samsun

Test 3.
Soil treatment test

Used virus

Pepper mild mottle virus **(PMMoV)**
Cucumber green mottle mosaic virus**(CGMMV)**

Used plant

PMMoV :
Tobacco **(cv. Samsun)**
CGMMV: ***Cucumber***

Method

Drenching treatment

Treatments

Viral sap : 100ml/pot

Result

Confirmed 4 weeks after inoculation by ELISA or eyes

Soil transmission- PMMoV & CGMMV

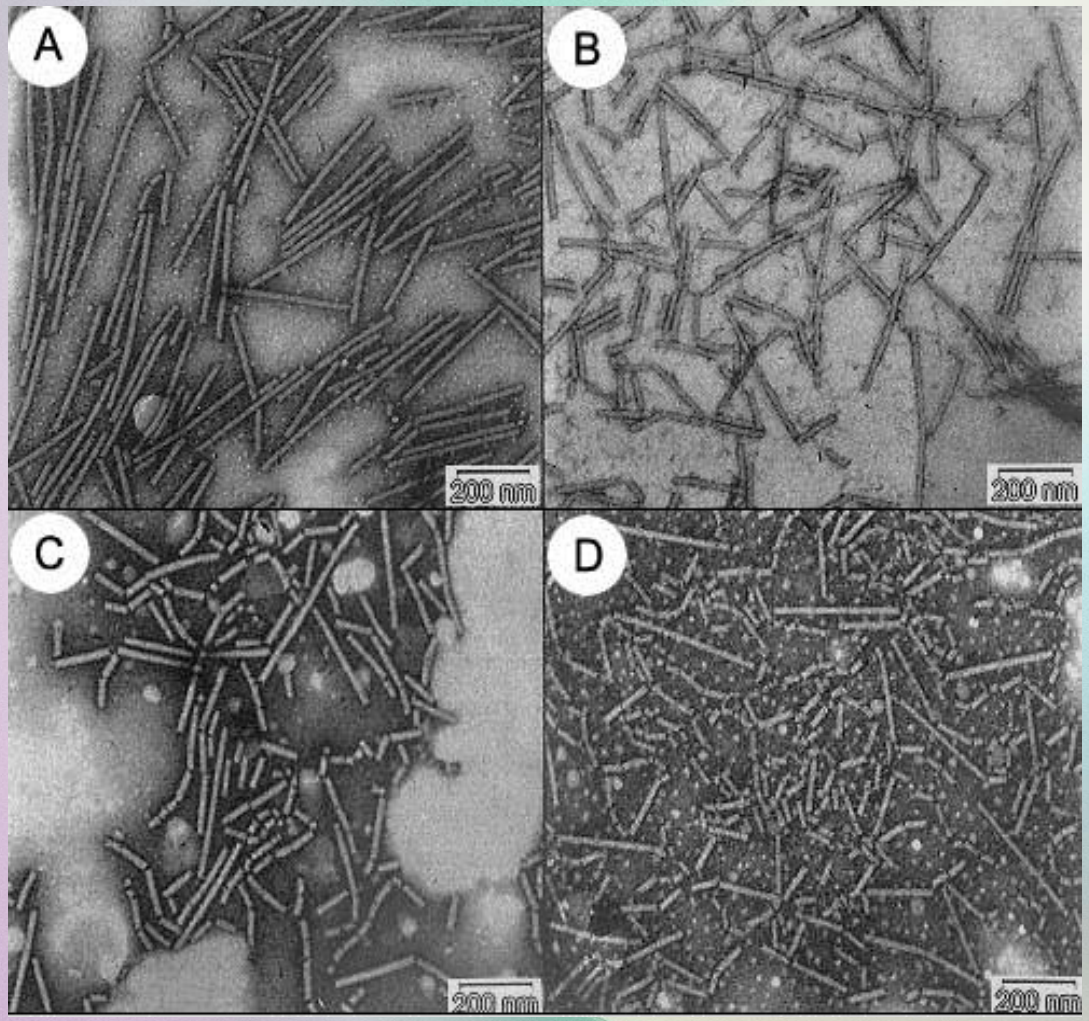
Table. Inhibitory activity of Qbyrus-1 against PMMoV in soil (on red pepper)

Week after inoculation	Concentration of extracts (mg/ml)[a)]	No. of infected/ tested plants	
		Qbyrus-1	Control
4	10	4/20	20/20
	5	8/20	
	1	14/20	

Table. Inhibitory activity of Qbyrus-1 against CGMMV infection in soil (on cucumber)

Week after inoculation	Concentration of extracts (mg/ml)[a)]	No. of infected/ tested plants	
		Qbyrus-1	Control
4	10	0/20	20/20
	5	0/20	
	1	7/20	

Electron micrographs of PMMoV particles in the absence(A) or presence of Qbyrus-1 (B,C,D)



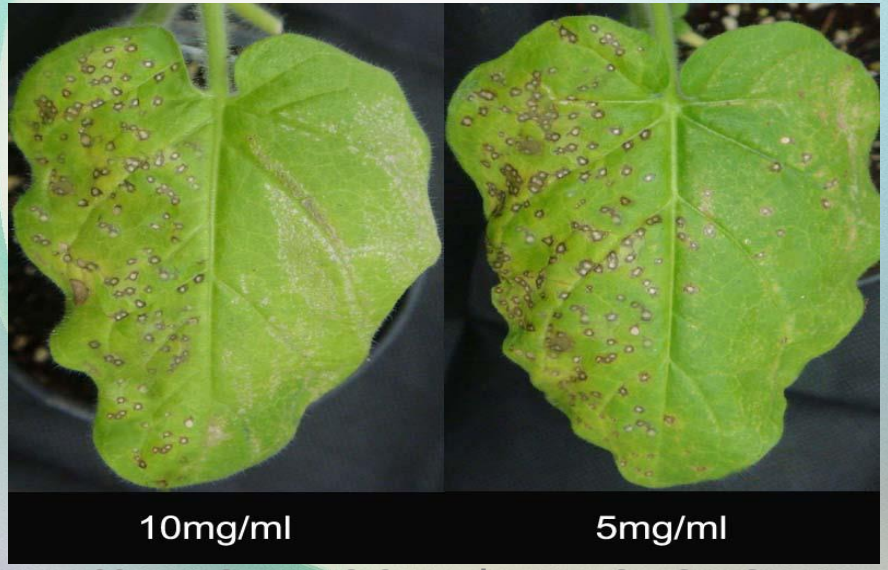
(A) purified virus particles
(B) 5mins after treatment
(C) 10mins after treatment
(D) 20mins after treatment

negatively stained with 2% PTA (pH 7.0). Bar=200nm.

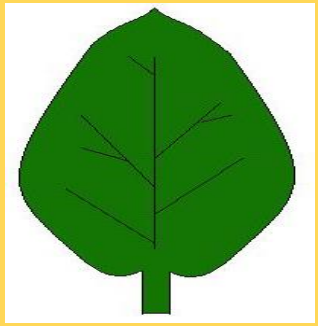
The effect on the infectivity of viral RNA

Table. Inhibition effects of Qbyrus-1 by application as mixture of PMMoV viral RNA and Qbyrus-1

Concentration of KN0912 (mg/ml)	No. of local lesions[a)]		Inhibition of PMMoV infectivity (%)[b)]
	Untreated	Treated	
10	257	0	100
5	276	14	94.9

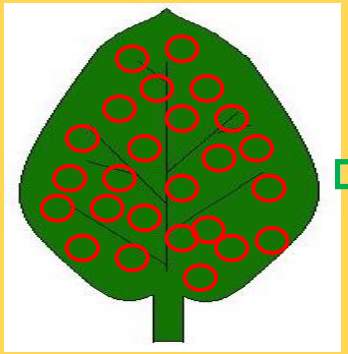


Antiviral activity against TMV : Leaf-disc method

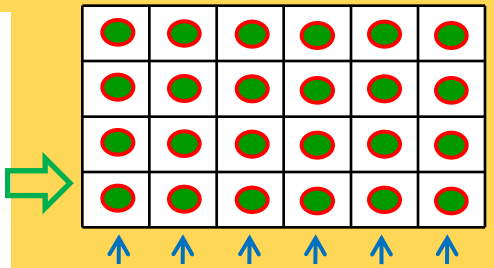


Virus infected leaf
(TMV-GFP inoculation)

Surface sterilize
(70 % ethanol, 2%
Sodium hypochlorite)



leaf disk



24 well culture plate(MS media containing antiviral agents)

after 1,2,3,4,5,6,7 dpi

-evaluated by observation expressions of GFP under UV light,
RT-PCR of viral RNA

Antiviral activity against TMV in systemic host plant : Tobacco cv. Samsun
Treated antiviral agents : A company's product and Qbyrus-1

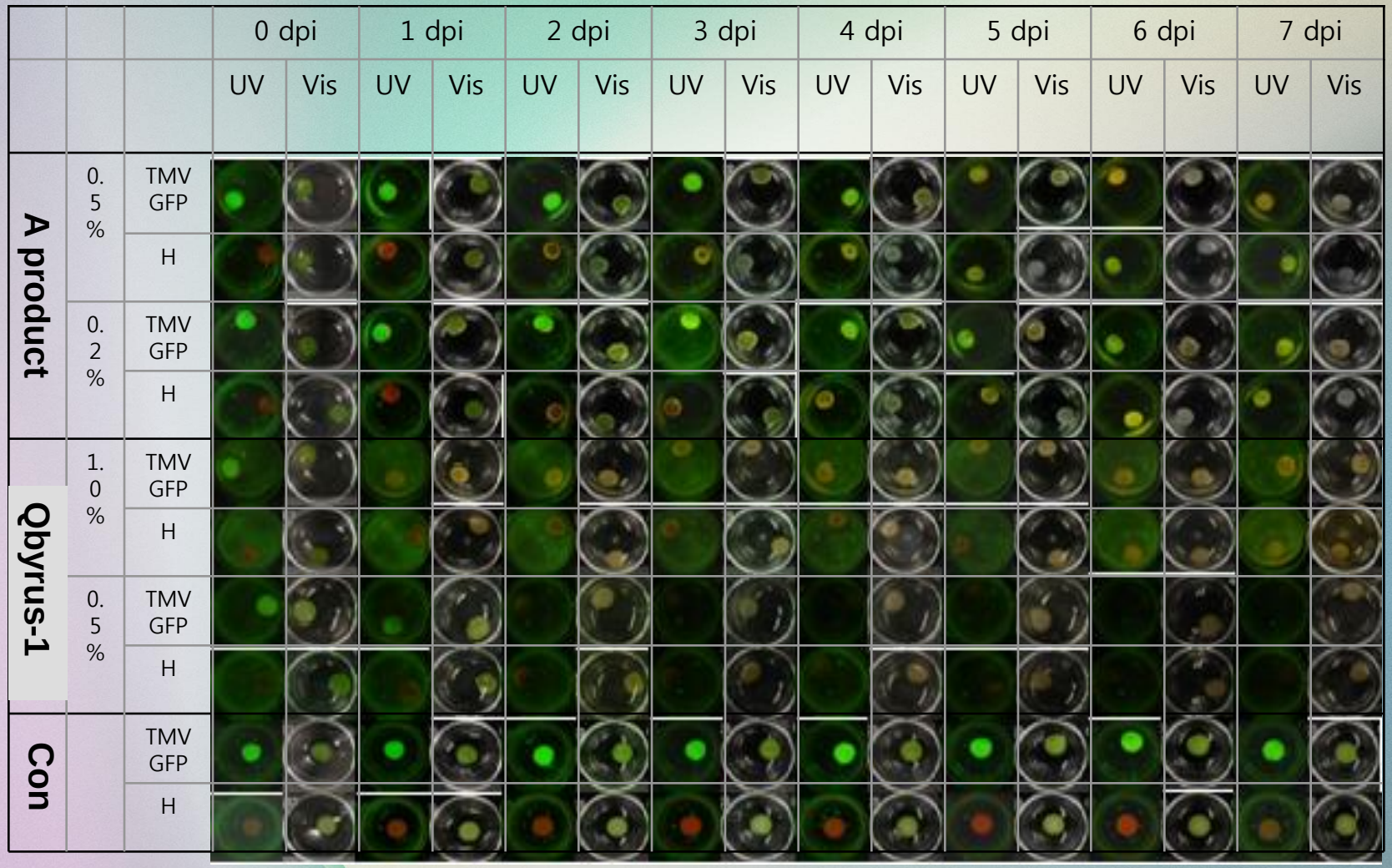


Fig. Antiviral activity against TMV-GFP using the Leaf-Disc Method

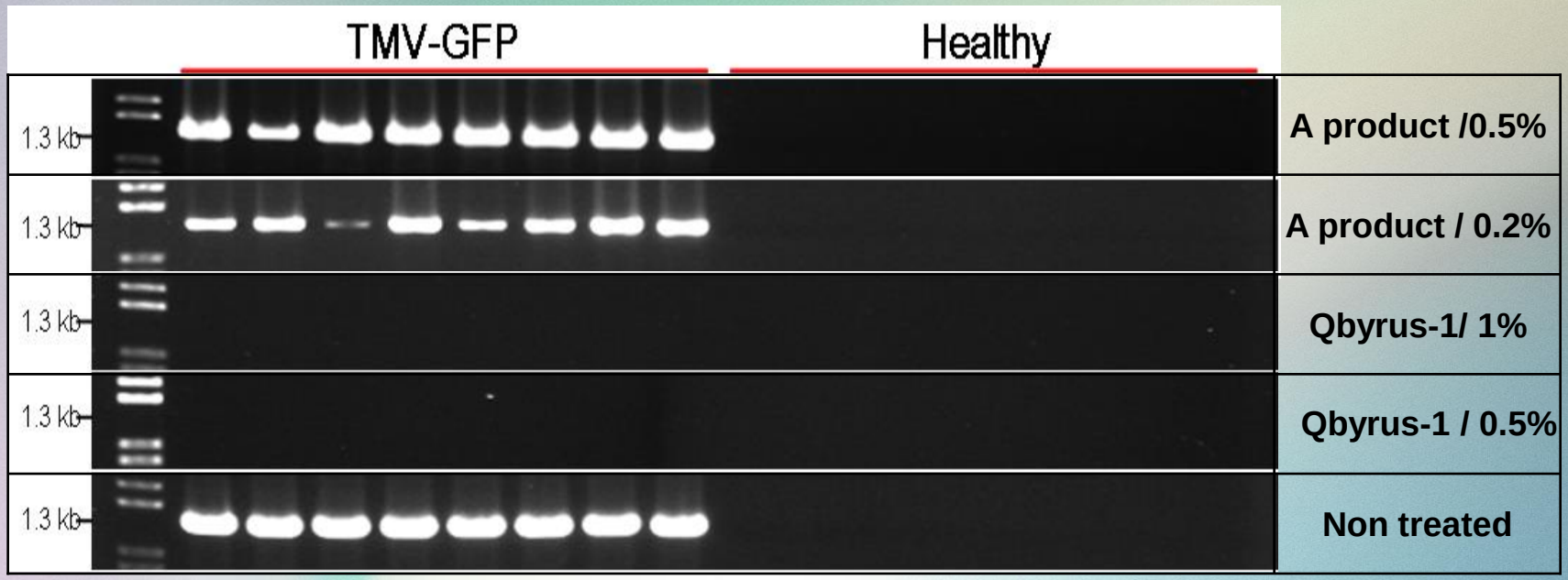
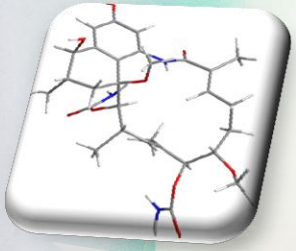


Fig. RT-PCR detection of TMV-GFP in leaf-discs using the Leaf-Disc Method

Leaf-discs treated with Qbyrus-1

- did not contain detectable viral RNA by RT-PCR
- The viral RNA of leaf-disc treated with Qbyrus-1 were degrade, RNase targeting the viral RNA exists in Qbyrus-1

Identification of antiviral components

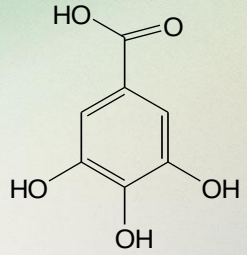


Compound 1 (Gallic acid / 3,4,5-trihydroxybenzoic acid)[1], C7H6O5, Mw: 170.12, mp: 253 (dec.)°C, UV (λmax, nm) (MeOH): 276 (3.01), IRv (cm-1, KBr): 3372, 1650 (COO), 1H-NMR (MeOH-*d4*) δ: 7.05 (2H, s, H-2,6), 13C-NMR (MeOH-*d4*) δ: 110.7 (C-2,6), 122.8 (C-1), 139.9 (C-4), 146.8 (C-3,4), 172.1 (COO), EI-MS: M+ = 170, m/z = 170(100), 153, 125, 79

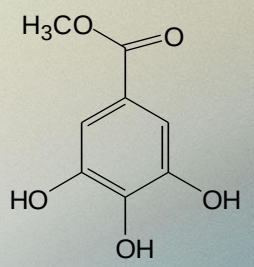
Compound 2 (Methyl gallate / Gallicin) [2], C8H8O5, Mw: 184.14, mp: 157°C, UV (λmax, nm) (MeOH): 276 (4.09), IRv (cm-1, KBr): 3360, 1695 (COO), 1620, 1536, 1440, 1374, 1H-NMR (MeOH-*d4*) δ: 3.80 (3H, s, COOCH3), 7.03 (2H, s, H-2,6), 13C-NMR (MeOH-*d4*) δ: 51.3 (OCH3), 109.0 (C-2,6), 120.4 (C-1), 138.7 (C-4), 145.5 (C-3,4), 168.0 (COO), EI-MS: M+ = 184, m/z = 153 (100), 125, 107, 79

Compound 3 (Methyl digallate *meta-, para-* isomer mixture) [3] C15H12O9, Mw: 336.23, mp: 212°C, UV (λmax, nm): 226 (4.8), 275 (4.15), IRv (cm-1, KBr): 3368, 1685 (COO), 1630, 1382, 1H-NMR (MeOH-*d4*) δ (*m*-isomer) 3.84 (3H, s, COOCH3), 7.10 (1H, d, J=2.13Hz, H-6')*, 7.23 (2H, d, J=2.04Hz,, H-2')*, 7.25 (H, d, J=1.73Hz, H-6), 7.39 (1H, d, J=1.71Hz, H-2); (p-isomer) 3.86 (3H, s, COOCH3), 7.20 (2H, s, H-2,6), 7.21 (2H, s, H-2',6')* 13C-NMR (MeOH-*d4*) δ (*m*-isomer) 52.5 (OCH3), 110.9 (C-2',6')*, 114.7 (C-6), 117.3 (C-2), 120.6 (C-1), 121.4 (C-1')*, 139.7 (C-4), 140.0 (C-4')*, 146.4 (C-3',5')* 146.6 (C-5), 147.6 (C-3), 166.3 (COO), 168.2 (COO)*; (*p*-isomer) 52.7 (OCH3), 109.9 (C-2, 6), 110.2 (C-2',6')* 120.5 (C-1')*, 128.5 (C-1), 132.5 (C-4), 140.1 (C-4')*, 146.3 (C-3',5')* 151.7 (C-3,5), 166.2 (COO), 168.3 (COO)*; FAB-MS: M+= 336, EI-MS: m/z = 184 [M-gallate] +, 170 [M-methyl gallate]+, 153(100), 125, 107, 79

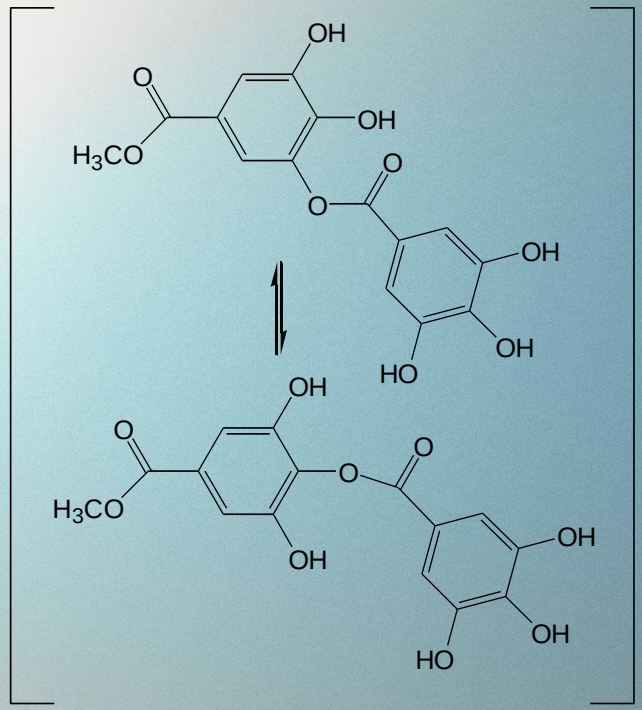
Signals (*) arising from depsidically linked galloyl groups.



1



2



3

Figure. Structure of isolated compounds. 1: gallic acid, 2: methyl gallate, 3: methyl digallate isomers (methyl m-digallate and methyl p-digallate)

Conclusions

The inhibitor named as Qbyrus-1 formulated from the gallnut extracts strongly inhibit TMV, PMMoV , CMV, CGMMV and ZYMV infection.

The results indicate that the Qbyrus-1 is a potent plant viral inhibitor that may be used to prevent the spread of plant virus infections in the field.

Commercialized Products

Biotech idea Group
B.I.G Co., Ltd.
www.big21c.co.kr

Website: www.big21c.co.kr

48-29 Munpyeong-dong, Daedeok-gu
Deajeon 306-220 Korea
T. +82 42 934 8155

Cabbage field of organic farming

Korea traditional house and garden

Thank you