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Antiviral potential of *Mirabilis jalapa* root extracts against groundnut bud necrosis virus

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Abstract
Tomato bud blight disease is a serious constraint in tomato cultivation and causes greater yield loss which is caused by *Groundnut bud necrosis* (GBNV). The aim of our study is to assess the antiviral activity of plants extracts *viz*., *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Andrographis paniculata* against GBNV in cowpea indicator host. The screening experiments were conducted in the glasshouse condition. The co-inoculation spray of 6% root extract of *Mirabilis jalapa* was effective in reducing the local lesions 1.50 lesions/cm^2 and virus titre (0.28±0.001) in cowpea plants compared to inoculated control plants where it was 6 lesions/cm^2 and 2.76±0.038 OD value in DAC-ELISA. Bioactive compounds *viz*., cyclohexane, 1, 3, 5- trimethyl-2-octadecyl-, dodecanoic acid in the root extract of *M. jalapa* were identified using Gas chromatography-mass spectrometry. Alcohol, halogens and phosphorus nature of the *M. jalapa* was identified using Fourier-transform infrared spectroscopy. Among the plant extracts 6% *Mirabilis jalapa* root extract showed the highest inhibitory activity against GBNV.

Keywords: *Groundnut bud necrosis*, cowpea, sap transmission, DAC- ELISA, GCMS and FT-IR

Introduction
Tomato (*Solanum lycopersicum*) is originated from Western South America and Central America. In India, tomato is grown in Tamil Nadu, Kerala, Karnataka, Maharashtra, Madhya Pradesh, and Uttar Pradesh. Tomato contains a large source of Vitamin C, E, and K; it helps to maintain the bones healthy and used in cosmetics, dermatology purpose because of the high amount of carotenoid. It contains basic nutrients for the heart functioning process (WHO, 2004) [35].

Tomato is affected by several diseases *viz*, early blight of tomato (*Alternaria* spp.) (Kemmitt, 2002) [13], anthracnose (*Colletotrichum* spp.) (Rashid *et al.*, 2015) [24], bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) (Holguin-Pena *et al*., 2016) [11], bacterial wilt (*Ralstonia solanaearum*) (Seleim *et al*., 2014) [27], bacterial spot (*Xanthomonas* spp.) (Borges *et al*., 2016) [3] and viral diseases caused by tomato spotted wilt virus (Dong *et al*., 2010) [5], tomato leaf curl New Delhi virus (Ruiz *et al*., 2015) [26], cucumber mosaic virus (Herrera-Vasquez *et al*., 2009) [10], tobacco mosaic virus (Liu *et al*., 2019) [16], tomato chlorosis virus and tomato infectious chlorosis virus (Tsai *et al*., 2004) [29]. Among these diseases, bud blight disease of tomato caused by *Groundnut bud necrosis virus* (GBNV) causes more economic losses (Mandal *et al*., 2012) [20].

Tomato is severely infected by Groundnut bud necrosis virus causing bud blight disease in tomato leads to severe yield loss with high disease incidence and showed necrosis symptoms on leaves and stems, chlorotic lesions on tomato fruit. Groundnut bud necrosis virus comes under the family *Tospoviridae*; genus *Ortho tospovirus* (Maes *et al*., 2018) [18]. Tospovirus seriously affects the production and productivity of tomato. GBNV is the positive ssRNA genome with spherical particles which contains three RNA in their genome *viz.*, LRNA, MRNA and SRNA (Adkins, 2000) [1].

Transmission of tospoviruses from plant to plant is caused by insect vector thrips belongs to the family; Thripidae, order; Thysanoptera, class; Insecta, phylum; Arthropoda in a circulative and propagative manner and also polyphagous nature. Three important thrips *viz.*, *Frankliniella occidentalis* (the Western flower thrips), *F. schultzei* (the cotton bud thrips), and *Thrips tabaci* (the onion thrips) were recorded to transmit the tospovirus and some other vectors also reported to transmit the virus *viz.*, *F. fusca*, *T. setosus*, *T. palmi*, and *Scirtothrips dorsalis*, and *F. intonsa* (Wijkamp *et a1*., 1994) [34].

Thrips transmitted tospoviruses cause the devastating yield to several economically important vegetable and flower crops. The specific characters of the thrips- tospovirus relationship is that only adults who acquired the virus at the first larval stage are able to transmit the virus. Both larval and adult thrips are transmitted to the tospovirus in tomato in a persistent manner (Manjunatha, 2008) [21]. Field management of thrips population which reduces virus transmission in tomato leads reduction of tospovirus infection in tomato.

Scientists used different methods to control viral diseases in plants. In that, botanicals were extensively studied for the antiviral effect against plant viruses. The antiviral activity of plant extracts *Thuja orientalis*, *Nigella sativa* L., *Azadirachta indica* and *Bougainvillea spectabilis* were evaluated against *Zucchini yellow mosaic virus* (ZYMV) infecting *Citrullus lanatus* (Elbeshehy, 2017) [6]. The *Mirabilis jalapa* anti-plant viral protein was reported by several researchers (Ikeda *et al*., 1987 [12], Vivanco *et al*., 1999 [31], Bolognesi *et al*., 2002 [2], Waziri, 2015) [32]. The antiviral activity of *Mirabilis jalapa* was demonstrated against bean common mosaic virus in bean plants (Elsharkawy, and El-Sawy, 2015) [7], and virus inhibiting agent was isolated from *Bougainvillea spectabilis* (Verma and Dwivedi, 1984) [30]. Similarly, the antiviral activity of *Andrographis paniculata* against the Chikungunya virus in humans was reported by Wintachai *et al.*, 2015 [29].

In our study, the antiviral principles of different plant extracts *viz*., *Bougainvillea spectabilis*, *Andrographis paniculata* and *Mirabilis jalapa* were used to manage the groundnut bud necrosis virus in the indicator host cowpea (*Vigna unguiculata*) cv. Co 7 was investigated.

Materials and Methods

Collection of GBNV infected samples

Groundnut bud necrosis virus infected samples showing bud blight, necrosis on the leaves and stems were collected from the tomato growing areas of Coimbatore district. The samples were used to check the antiviral activity in cowpea cultivar var (Co-7) under insect-proof condition in glasshouse. The necrotized young leaves were collected from the field was thoroughly washed with running tap water to remove the dust and were slightly dried in tissue paper to remove the excess water. After that, the leaves were grounded in sterilized, pre-chilled pestle and mortar by adding (1:1 w/v) of 0.1 M sodium phosphate buffer (pH 7.1-7.2) containing β-mercaptoethanol at 0.1%. The whole macerating procedure was carried out in cold conditions. The macerated sap was filtered through dual form of muslin cloth. The GBNV sap extract was further used in the inoculation on cowpea. Treatments *viz*., pre-inoculation (AVPs sprayed at 24 hours before virus inoculation), co-inoculation (AVPs and virus were inoculated simultaneously), post-inoculation (AVPs sprayed at 24 hours after virus inoculation), inoculated control and untreated uninoculated control were maintained. Each treatment was replicated thrice with nine plants in each replication. The lesion count was recorded in all the treatments after 4 days of inoculation. The lesions numbers per cm^2 area recorded and analysed by CRD ANOVA with three replications.

Antiviral principles extraction

Mirabilis jalapa roots were collected from the Horticultural Research Station, Thadiyankudisai (10.2995° N, 77.7118° E) Kodaikanal, Dindigul district. Roots were sliced into small pieces and shade dried completely. After that, it was grounded as a fine powder using the blender mixer and used for the study. Then, the *Andrographis paniculata* and *Bougainvillea spectabilis* leaves were collected from TNAU campus (11.0152 °N, 76.9326 °E), Coimbatore (Fig. 1). In the same way, leaves weighed according to the concentration and water (w/v) was added and macerated in the mixer grinder and used for the screening. The antiviral principles (plant extracts) with a uniform concentration of 6% were used for spraying in cowpea plants under glasshouse conditions.

Fig 1: Antiviral principles containing plants collected from the different areas for screening. a) *Mirabilis jalapa* plant; b) Roots of *M. jalapa*; c) root pieces of *M. jalapa*; d) *Andrographis paniculata* plant and e) *Bougainvillea spectabilis*.

Quantification of virus titre in treatments by DAC-ELISA

GBNV titre in the leaf samples collected from all the treatments were checked based on the protocol given by Singh *et al*., 2018 [28] and Direct antigen coating ELISA was carried out in 96 well polystyrene plate. The OD value was recorded using EPOCH/2- microplate reader (BioTek, USA.) at 405 nm wavelength. The mean of OD value of three replicates were used to obtain standard error of the samples.

Sample preparation for GCMS and FT-IR

The *Mirabilis jalapa* root extract powder 10g was taken in the conical flask and added with 100 ml of ethyl acetate, kept at 37°C for overnight at 150 rpm in shaker cum incubator. Subsequently, the extract was separated using separating funnel and the fractions were condensed using a rotatory vacuum flash evaporator at 40ºC for 150 rpm 45 mins (Hajji *et al*., 2010 [9], Gogoi *et al*., 2016) [8]. Afterward, the fraction was poured into the petri plates and kept for drying. Then, the fractions were scrapped using HPLC grade methanol and filtered using a 0.02µm syringe filter. Finally, the samples were analysed in GCMS and FT-IR analysis at Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Further, identification of the compounds present in the plant extracts (GCMS) was compared based on the retention time and mass spectral fragmentation patterns with those stored on the computer library; National Institute of Standards and Technology (NIST) library.

Results

Efficacy of plant extracts against GBNV in indicator host cowpea

In the efficacy of plants extracts study, the result showed that, a significant reduction in the number of lesions per cm^2 in the cowpea plants treated with co- inoculation spray of *Mirabilis jalapa* (6%) (1.503 lesions/cm^2) compared to inoculated control plants (6.5 lesions/cm^2) (Fig.2). Followed by pre-

inoculation spray with the least number of lesions 1.76 per cm^2. The co-inoculation spray of *Andrographis paniculate* (6%) treated plants showed 2.2 lesions per cm^2. *Bougainvillea spectabilis* (6%) showed lesions from 3.57 to 4.51 lesions/cm^2. Inoculated control plants showed the maximum of 6 lesions per cm^2. The results showed that the simultaneous application of *Mirabilis jalapa* root extract (6%) inhibited the lesion formation and reduced the disease intensity. Less number of lesions was observed in the plants treated with *M. jalapa* (6%) (Co-inoculation) whereas a greater number of lesions were observed in the inoculated untreated control plant (Fig.3).

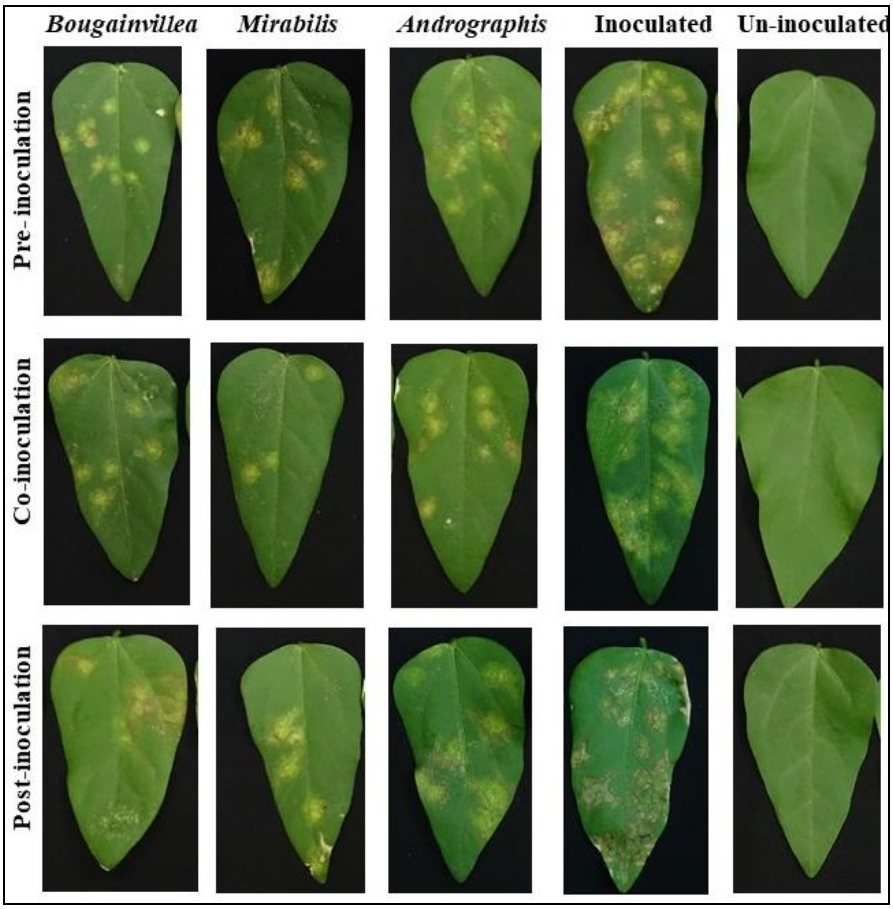

Fig 2: Antiviral activity of plants extracts against GBNV in indicator host cowpea under glass house condition with pre, co and post inoculation.

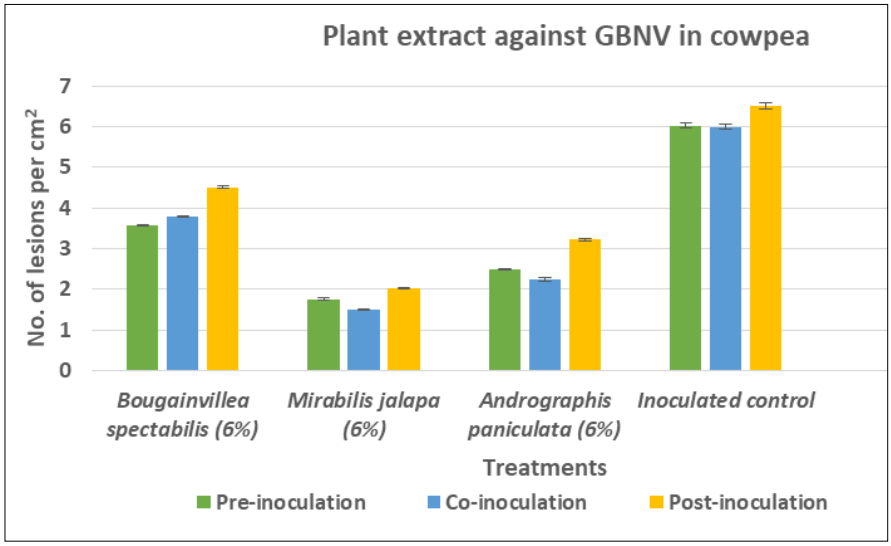

Fig 3: Plants extracts against GBNV inhibition of number of lesions over the inoculated control plants.

Estimation of virus titre in cowpea plants treated with plant extracts

The GBNV titre was assessed in the cowpea plants treated with plant extracts by DAC-ELISA. The virus titre was less in the plants in the co-inoculation of *M. jalapa* (6%) with OD value about 0.28± 0.001 at 405 nm followed by co-inoculation spray of *Andrographis paniculata* (0.30± 0.001). Pre-inoculation spray of *Mirabilis jalapa* (6%) and *Andrographis paniculata* (6%) exhibited OD value 0.44± 0.007 and 0.46± 0.001 respectively. Untreated inoculated control plants showed the highest OD value ranges at 2.76± 0.038 (Fig.4).

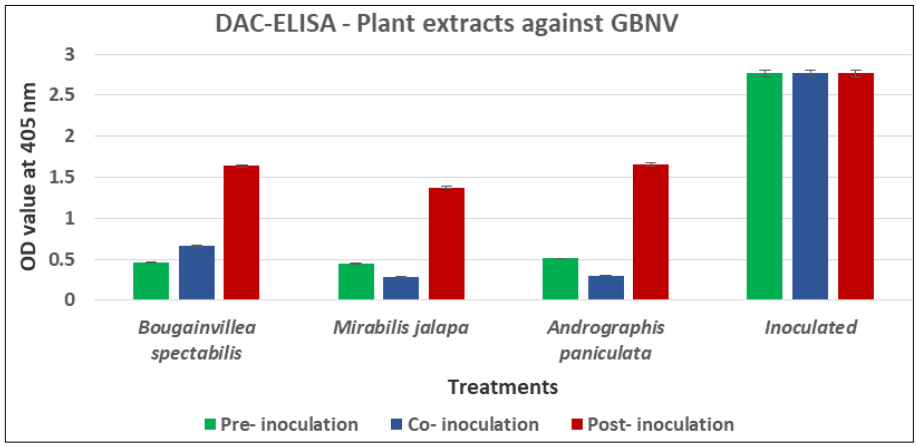

Fig 4: GBNV titre over the inoculated control in the DAC-ELISA, the cowpea plants treated with plants extracts.

Metabolic profiling of root extract of *Mirabilis jalapa*

GCMS chromatograph of *M. jalapa* is represented in Fig.5. Twenty-four chemical components were identified from the methanolic extract of root extract of *M. jalapa* (Table.1). The major chemical components are 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, l-Alanine, N-methoxycarbonyl-, butyl ester, Cyclohexane, 1,3,5- trimethyl-2-octadecyl-, Dodecanoic acid, N-(2-Methylbutyl)(2E,4E,8Z,10E)- dodecatetraenamide, Pentadecanoic acid, Palmitoleic acid, 2-Methoxy-4-vinylphenol, (S)-5-Hydroxymethyl-2[5H]-furanone, Hexahydro-5H-imidazo[5,1-b:4,3-b']bisthiazole, 1,6-Anhydro-à-d-galactofuranose, 2-Oleoylglycerol, 2TMS derivative and Glycerol 1-palmitate.

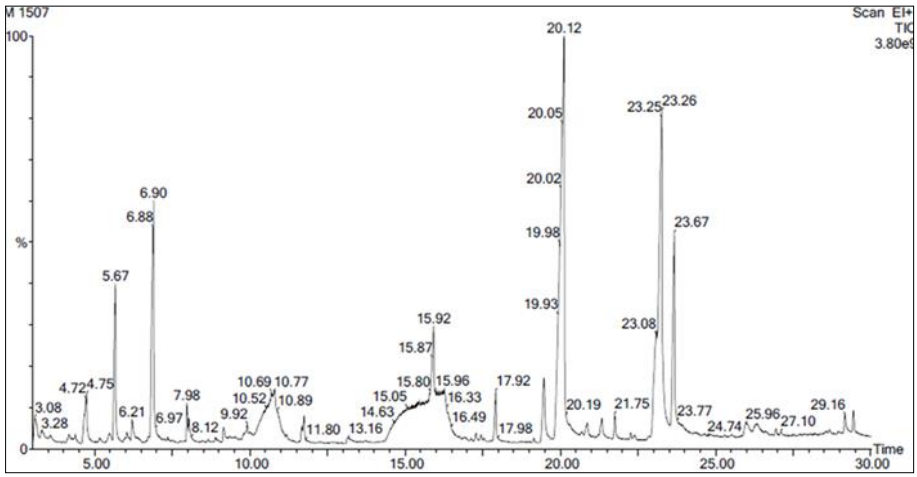

Fig 5: GC-MS chromatograph for the root extract of *Mirabilis jalapa*

Table 1: GC-MS analysis of *Mirabilis jalapa* root extract.

S. No	Retention time	Peak area percentage	Compound name	Molecular weight (g/mol)	Molecular formula
1.	3.299	0.857	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	144.12	$C_6H_8O_4$
2.	3.579	0.460	(+)-N (2)-Ethyl-4-methyl-1,2-pentanediamine	144.26	$C_8H_{20}N_2$
3.	5.494	0.240	l-Alanine, N-methoxycarbonyl-, butyl ester	493.3	$C_{22}H_{24}INO_4$
4.	6.050	0.159	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	285.13	$C_{12}H_{13}N_7$
5.	6.210	0.505	(S)-5-Hydroxymethyl-2[5H]-furanone	114.1	$C_5H_6O_3$
6.	6.370	0.163	5-Oxotetrahydrofuran-2-carboxylic acid, ethyl ester	158.15	$C_7H_{10}O_4$
7.	7.090	0.162	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	124.22	C_9H_{16}
8.	7.975	0.457	2-Methoxy-4-vinylphenol	150.177	$C_9H_{10}O_2$
9.	8.030	0.580	4-Heptanol, acetate	116.2	$C_7H_{16}O$
10.	9.156	0.372	Dimethyl(bis[(2Z)-pent-2-en-1-yloxy])silane	146.38	$C_6H_{18}Si_2$
11.	9.526	0.132	Butanal, 2-methylene-, diethylhydrazone	154.25	$C_9H_{18}N_2$
12.	9.916	0.477	Octanoic acid, 2,4,6-trimethyl-, methyl ester	214.348	$C_{13}H_{26}O_2$
13.	10.001	0.284	Hexahydro-5H-imidazo[5,1-b:4,3-b']bisthiazole	188.3	$C_5H_4N_2S$
14.	10.781	7.879	d-Gluco-heptulosan	210.18	$C_7H_{14}O_7$
15.	11.747	0.441	Dodecanoic acid	201.31	$C_{12}H_{24}O_2$
16.	13.177	0.330	1,6-Anhydro-à-d-galactofuranose	162.14	$C_6H_{10}O_5$
17.	15.918	3.090	Tetradecanoic acid	229.36	$C_{14}H_{28}O_2$
18.	16.919	0.159	N-(2-Methylbutyl)(2E,4E,8Z,10E)-dodecatetraenamide	68.12	C_5H_8

19.	17.919	1.000	Pentadecanoic acid	242.4	$C_{15}H_{30}O_2$
20.	19.475	1.863	Palmitoleic acid	254.41	$C_{16}H_{30}O_2$
21.	21.341	0.581	cis-10-Heptadecenoic acid	282.5	$C_{18}H_{34}O_2$
22.	23.927	0.164	2-Oleoylglycerol, 2TMS derivative	500.9	$C_{27}H_{56}O_4Si_2$
23.	26.608	0.183	Cyclohexane, 1,3,5-trimethyl-2-octadecyl-	124.22	C_9H_{16}
24.	29.164	0.404	Glycerol 1-palmitate	330.5	$C_{19}H_{38}O_4$

FT-IR spectrum of *Mirabilis jalapa*

Secondary metabolites of *M. jalapa* methanolic extract was analyzed in the FT-IR spectrum (Fig.6). Absorption bands and their organic compounds obtained from the metabolites were given in the table. In the spectrum, the absorption wavelength varied from 440-3400 cm^{-1}. A strong stretch alcoholic R-CH2-OH group was found in wavelength of 3400-3200 cm^{-1} with OH bond. Halogen classification variable- strong stretches were observed in the wavelength 1300-900 cm^{-1} in the C-F group, strong stretches 600-500 cm^{-1} and 610-485 cm^{-1}observed in C-Br and C-I group respectively. Phosphorus compound has strong stretches in the wavelength 1025-870 $cm^{-1}$and 580-440 cm^{-1} P-O-P and P-Cl group respectively. In the wavelength, 1100-1000 cm^{-1} and 1020-1010 cm^{-1} variable to strong stretches of silicon compound observed in the Si-O-Si group. Ph-CHR-OH group of alcohols were observed in the wavelength of about 1350-1260 cm^{-1}(OH bond) and 1075-1000 cm^{-1}(C-O bond) strong stretches were obtained. Variable alcoholic stretches were obtained in the wavelength of about 3400-3200 cm^{-1}(Table.2).

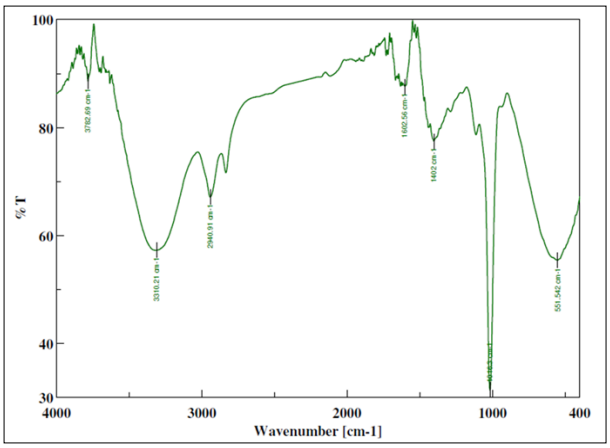

Fig 6: FT-IR spectrum of root extract of *Mirabilis jalapa*.

Table 2: FT-IR analysis of *Mirabilis jalapa* root extract.

S. No	Classification	Group	Bond	Wavelength cm^{-1}	Intensity	Mode	Notes
1.	Halogens	C-F	C-F	1300-900	Variable- Strong	Stretching	
2.	Phosphorus compound	P-O-P	P-O-P	1025-870	Strong	Stretching	
3.	Silicon compound	Si-O-Si 6 member ring	Si-O-Si	1020-1010	Variable- Strong	Stretching	Cyclic trimer
4.	Silicon compound	Si-O-Si		1100-1000	Variable- strong	Stretching	Open chain
5.	Alcohols	$R-CH_2-OH$					
			OH	3400-3200	Variable	Stretching	Hydrogen bonded, broad peak
			OH	1480-1410	Medium- weak	deformation	
			C-O	1075-1000	Strong	Stretching	
6.	Alcohols	Ph-CHR-OH	OH	3400-3200	Variable	Stretching	Hydrogen bonded, broad peak
			OH	1350-1260	Strong	deformation	
			C-O	1075-1000	Strong	Stretching	
7.	Halogens	C-Br	C-Br	600-500	Strong	Stretching	
8.	Halogens	C-I	C-I	610-485	Strong	Stretching	
9.	Phosphorus compound	P-Cl	P-Cl	580-440	Strong	Stretching	
10.	Silicon compound	Si-Cl	Si-Cl	550-470	Strong	Stretching	

Discussion

Tomato infected with groundnut bud necrosis virus resulted in severe yield loss and much more disease incidence (Mandal *et al*., 2012) [20]. This virus infects particularly in seedling and flowering stage of the crop, which ends with bud blight. Nowadays, some of the medicinal plants, as well as antiviral proteins, were used for the management of plant virus by several researchers (Narwal *et al*., 2001 [22], Bolognesi *et al*., 2002 [2], Renukadevi *et al*., 2004 [25], Deepthi *et al*., 2007 [4], Elsharkawy and El-Sawy, 2015 [7], Kusumawati *et al*., 2017 [15], Elbeshehy, 2017) [6]. Many plants naturally possess bioactive compounds to protect the plants from several diseases by enhancing resistance capability in the plants and also the plant extracts are nonphytotoxic, not harmful to the environment and unhazardous. In our study, we have used *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Andrographis paniculata* which contains antiviral proteins that protected the plants against GBNV under glasshouse condition. The application of plant extracts promotes the systemic acquired resistance in plants.

Numbers of lesions were reduced in the cowpea plants treated with a co-inoculation spray of *M. jalapa* root extract (6%). In the same way, Elsharkawy *et al*. (2015) [7], used *Mirabilis jalapa* root extracts recorded the reduction in number of lesions in the plants treated with plant extracts. Likewise, Ribosome-inactivating proteins (RIP) were reported in *Mirabilis jalapa* by Bolognesi *et al*., 2002 [2], which inhibits the translation of the virus and also called as MAP (*Mirabilis* antiviral protein). In the same way, Blackeye cowpea mosaic disease in cowpea plants reduced the disease incidence up to 7% in the glasshouse condition and 31% in field condition when the seeds and seedlings were treated with *B. spectabilis* and 48% disease reduction in *M. jalapa* leaf extract (Prasad *et al*., 2007) [23].

Deepthi *et al*. (2007) [4] reported that the reduction of the number of lesions in *N. glutinosa* plants treated with plant extracts, particularly *Thuja occidentalis* effective against *Tobacco mosaic virus* (TMV) and *Tomato mosaic virus* (ToMV).

In our study, also pre-inoculation spray of *M. jalapa* root

extract (6%) reduced the lesion level in a significant level. Besides, Elbeshehy, 2017 [6] reported the inhibitory activity of medicinal plant *Thuja orientalis* against *Zucchini yellow mosaic virus* in watermelon and recorded the pre and post-inoculation of plants extract were effectively reduced the symptom expression in watermelon. Moreover, the induction of disease resistance was reported against tobamovirus in *N. glutinosa* by reducing the number of lesions in the plants treated with *Bougainvillea spectabilis* (Madhusudhan *et al*., 2011) [17]. *Bougainvillea* showed low activity against GBNV in cowpea, then *M. jalapa* and *A. paniculate.* It may infer that; application of root extract reduced the virus multiplication by induced systemic resistance. Earlier, Verma and Dwivedi, 1984 [30], reported the virus inhibiting agent from the *Bougainvillea spectabilis* in post-inoculation and also recorded the systemic induced resistance against tobacco mosaic virus.

In our study, we quantified less GBNV virus titre in the co-inoculation of *M. jalapa* treated plants in DAC- ELISA compared to the inoculated control plants. Similarly, Renukadevi *et al*. 2004 [25] reported that the pre-application of *M. jalapa* and *H. cupanioides* inhibited the lesion formation and induced phenols, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase activity in the plants.

In our study, various bioactive compounds *viz*., Cyclohexane, 1,3,5-trimethyl-2-octadecyl-, Dodecanoic acid, N-(2-Methylbutyl) (2E,4E,8Z,10E)- dodecatetraenamide, Pentadecanoic acid, Palmitoleic acid were identified by GCMS chromatography in the effective treatment root extract of *M. jalapa.* These bioactive compounds present in the root extract of *M. jalapa* may responsible for the antiviral activity of GBNV in cowpea.

Mirabilis jalapa antiviral protein was reported against the tobacco mosaic virus by Ikeda *et al.*, 1987 [12]. Similarly, Kubo *et al*., 1990 [14] recorded the antiviral activity of *M. jalapa* against TMV in *Nicotiana* var *xanthi*, where 100% inhibition was obtained. Vivanco, 1999 [31] reported the presence of ribosome-inactivating protein Mirabilis antiviral protein (MAP) which inhibited potato virus X, potato virus Y, potato leaf roll virus, and potato spindle tuber viroid in the roots of *M. jalapa*. The presence of bioactive compound Andrographolie in *Andrograpghis paniculata* has a hepatoprotective activity (Maiti *et al*., 2009) [19] and against *Chikungunya virus* (CHIKV) was reported by Wintachai *et al*., 2015 [29].

The present study strongly infers that *M. jalapa* root extract spray (6%) effectively suppress GBNV in indicator host cowpea.

Conclusions

This *M. jalapa* root extract will definitely check GBNV incidence in tomato field. Further research was intensified to verify the role of *M. jalapa* root extract in the control of various plant viruses under field condition.

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