The Pharma Innovation Journal 2017; 6(11): 574-578

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 574-578

www.thepharmajournal.com
Received: 25-09-2017
Accepted: 27-10-2017

Vasanthi VJ
Assistant Professor, Department of Plant Pathology, Adhiparasakthi Horticultural College, Kalavai, Tamil Nadu, India

Samiyappan R
Professor (Retd), Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Vetrivel T
Assistant Professor, Dept of Horticulture, Dhanlakshmi Srinivasan College of Agriculture, Perambulur, Tamil Nadu, India

Correspondence
Vasanthi VJ
Assistant Professor, Department of Plant Pathology, Adhiparasakthi Horticultural College, Kalavai, Tamil Nadu, India

Development of a new chitin based bio-formulation of *Pseudomonas fluorescens* and a natural insecticide *(Vitex trifolia)* against Indian Tomato Leaf Curl Virus (iTLCV) and its whitefly vector

Vasanthi VJ, Samiyappan R and Vetrivel T

Abstract
The present study focused on different methods of application of talc based powder formulation of *Pseudomonas fluorescens* strains and strain mixtures with chitin to seed, soil and foliage or as a seedling dip followed by two foliar sprays of 2% leaf extract of *Vitex trifolia* (Natural insecticide), which significantly reduced whitefly vector nymphs and adults and also reduced the incidence of iTLCV in tomato plants both in glasshouse and field conditions. Further there was an increase in growth promotion in PGPR tomato plants. DAS ELISA tests showed low concentration of viral antigen in PGPR treated tomato leaves and also in nymphs and adult whiteflies. An ecofriendly approach to manage the viral disease and optimization of tomato yield has been initiated.

Keywords: iTLCV, Bemesia *tabaci*, *Pseudomonas*, *Vitex*, Chitin, DAS ELISA, Tomato

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable grown worldwide. The major constraint in tomato production is insect vector- transmitted virus diseases. Effective insecticidal control for insect-borne disease is quite problematic as plant disease vectors are highly mobile insects and colonize fields rapidly. In addition, even low numbers of insects may result in high field incidence of disease and further, insecticides were completely ineffective to prevent a serious epidemic of cucumber mosaic virus (CMV) transmitted by aphids in a non-persistent manner on tomato (Sikora *et al*. 1998) [11]. Microbe-induced resistance can be an alternative strategy for management of insect-transmitted diseases. Ross (1961) reported that tobacco plants exhibited "systemic acquired resistance" following local infection with tobacco mosaic virus.

Fluorescent pseudomonads, non-pathogenic rhizobacteria, are among the most effective biological control agents against soil-borne plant pathogens (Kloepper and Schroth 1978). PGPR are known to induce resistance against fungal, bacterial and viral diseases Induction of plant defenses through the use of plant growth promoting rhizobacteria (PGPR) is the current mechanism in the management of viral disease (Maurhofer *et al.* 1994; Raupach *et al.* 1996; Murphy *et al.* 2000; Zehnder*et al*. 2000; Kandan *et al*. 2002, Vasanthi *et al*., 2010) [10, 17, 11, 24].

Vitex trifolia belongs to genus *vitex* and family *Verbenaceae* there are approximately 270 known species of shrubs and trees. *Vitex trifolia* possess larvicidal, insecticidal properties in nature and also wound healing, anti-HIV, trypanocidal, antimicrobial activity (Hossain *et. al*., 2001) [4], antibacterial (Kannathasan, *et. al*., 2011) [7] and anticancer activity (Vasanthi *et al*., 2014) [13]. It is also used as antibacterial, anti-inflammatory, antipyretic agent. Molecular and crystal structures of methyl-*p*-hydroxybenzoate isolated from the leaves of *Vitex trifolia*, proved to be a potential mosquito larvicidal compound (Kannathasan. *et. al.*, 2008) [6]. Chemical control of plant diseases is usually expensive and may have a negative impact on the environment and on public health. Biological control makes management of plant diseases less dependent on the use of high-risk chemicals and it is environmentally friendly.

Materials and methods
Fluorescent pseudomonads were isolated from rhizosphere soils of different crops using King's medium B (KMB) and identified as *P. fluorescens* and *P. putida based* on gelatin liquefaction, arginine dihydrolase, nitrate reduction and various carbon source utilization

(Hildebrandt *et al*., 1992). The fluorescent pseudomonads isolates Pf1, VPT 10 belonged to *P. fluorescens* group. The cultures were maintained on KMB agar slants (King *et al*., 1954) [8]. The bacterial isolates were multiplied in KMB broth for 48 h and bacterial cells were collected by centrifugation at 8000*g* for 10 min and suspended in $MgSO_4$ and population was adjusted to 3×10^8 colony forming units (CFU) as measured spectrophotometrically (Thompson, 1996).

Chitin amendments with talc-based formulations

Five grams of crab shell chitin (Sigma, USA) were slowly added to 100 ml of cold 0.25 N HCl with vigorous stirring and kept overnight at 48C. The mixture was filtered through glass wool into 200 ml of ice cold ethanol at 48C with rapid stirring. The resultant chitin suspension was centrifuged at 10,000 rpm for 20 min and the chitin pellets were washed repeatedly with distilled water until the pH became neutral (Roberts and Selitrennikoff,1988). The concentration was adjusted to 10 mg/ml and added to KBB (1% v/v). Then the liquid medium (containing chitin) was autoclaved and the respective *P. fluorescens* isolates were allowed to grow in the medium for 48 h. The talc-based formulation containing chitin was prepared as described above.

Preparation of extracts from leaves of V*itex trifolia*

The fresh leaves of *Vitex trifolia* were collected from well grown trees of National Siddha Institute, Chrompet. TN. 1kg of fresh *Vitex trifolia leaves* were collected and ground well with water, using mortar and extract was filtered through muslin cloth and diluted (1: 5) and then used for spraying, after mixing with teepol. Water extract contained carbohydrates, flavonoids and Terpenoids. (Nancy *et al.*, 2014) [13].

Insect Vector studies in Glasshouse -Maintenance of Tomato seedlings

Tomato seeds were sown in Mud pots filled with farmyard manure and 20, 30, 45 DAS plants were selected for virus inoculation through whitefly vectors. (Ragupathi, 1995) [16].

Maintenance of viral inoculum

The iTLCV infected tomato plants were collected from nearby farmer's field and inoculated into healthy tomato plants using whiteflies collected from infected plants and the pots were kept in insect proof rearing cages in glasshouse and maintained throughout the experiment.

Whitefly vector rearing cages.

Whitefly vector are collected from Brinjal and cotton fields by sucking with a help of an aspirator from the lower surface of leaves and blown in to tomato plants kept in rearing cages for 48 hrs. They are continuously maintained in cages and made viruliferous so that they can infect PGPR treated tomato and control plants in glasshouse.

Release of viruliferous whitefly vectors

The whiteflies become viruliferous after feeding on infected tomato plants and they were released on PGPR treated tomato plants using an aspirator. An inoculation period of 24 hrs is given to viruliferous whiteflies to inoculate the virus into PGPR treated plants. All the tomato plants were infected with iTLCV by these whiteflies.

Seed Bacterization

Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivar CO3 were surface-sterilized with 1% sodium hypochlorite for 30 s and then rinsed in sterile distilled water and dried under a sterile air stream. Ten ml of bacterial inoculum containing 3×10^8 CFU/ml was added to Petri plates. To this, 100 mg of carboxyl methyl cellulose was added as adhesive material. Ten gram of seeds were soaked in 10 ml of bacterial suspension for 12 h. Then, the bacterial suspension was drained off and the seeds were dried overnight in sterile Petri plates.

Mixtures of bacterial strains

Were produced by mixing equal volumes of bacterial suspension of two strains sown separately. Inoculum of the two strains was then mixed with talc powder and the formulation prepared as described before.

Field studies

The talc-based formulations of single and mixtures of *P. fluorescens*strains with or without chitin were also evaluated under field conditions. A field area (Red soil, pH 7.0; previous crop, sorghum (*Sorghum vulgare*)) at kiliyanurpatti, Tamil Nadu, endemic with iTLCV was selected and the trials were conducted in 2014 and 2015. The weather conditions were conducive for the rapid spread of iTLCV in tomato by whiteflies. The trial was conducted as a randomized complete block design with three replicate plots treatment. The plot size selected was around 4x 3.5 m^2.

Seed treatment, seedling root dip, soil application and foliar spray of *P. fluorescens*strains and also foliar spray of *Vitex* leaf extract in specific treatments (T3, T4) were done as described before. No pesticides were applied in PGPR treatments except natural pesticide *Vitex trifolia* leaf extract (2 %) and the area was hand-weeded. The crop was irrigated every 6 days. Natural incidence of iTLCV was recorded 20, 30, 45 and 60 days after planting. The effects of *P. fluorescensn* on growth (plant height, plant biomass) and yield (fruiting cluster/plant, average fruit weight, number of fruits/plant and total yield) were also recorded. The data was analyzed statistically (Duncan Multiple Range Test).

Disease incidence and severity

Disease incidence (percentage of infected plants) were assessed by examining the plants for iTLCV symptoms. Plants were also scored individually for symptom severity using a 0-4 rating scale (0=No symptoms and 4=Severe symptoms) and severity for each plot was calculated. Records on disease incidence and severity level were taken at weekly or bi weekly intervals, starting one week after transplanting. Data on disease incidence were transformed to arcsine and analyzed using the analysis of variance procedure. (Ragupathi, 1995) [16].

Counting the insect vector numbers

Adult whiteflies were counted on a middle leaf per plant and their population was expressed on a per 100-leaf basis. Variation due to seasonal population changes, numbers of whiteflies were expressed as percentages of the respective untreated check and analyzed after transformation to arcsine.

Das Elisa

A standard double antibody sandwich (DAS) ELISA method was adopted to detect the viral antigen as described by

Mumford *et al*. (1994). Microtitre plates (Tarsons Pvt. Company, India) were coated with the capture antibody, a iTLCV specific polyclonal antiserum were obtained from Dr. G. Thottaphilly, ITTA, SA, diluted with 0.05 M sodium carbonate buffer (pH 9.6) to a dilution of 1/1000, with 100 mL added well as described by Mumford *et al.* (1994). Absorbance was determined at 405 nm using a Bio-Rad model 3550 (Microplate reader, USA).

Results

The results revealed for an effective management of whiteflies nymphs and adults which transmitted iTLCV in Tomato. Talc-based formulations of PGPR strains along with chitin were applied to seed, seedling and soil, foliar followed by two foliar sprays with *Vitex trifolia* extract (instead of PGPR strains in T_3 and T_4) at 15 days interval was considered as the best treatment (Pf1+Vpt 10+ Chitin + *Vite*x leaf extract) along with very low disease incidence and percent disease reduction over control was recorded as 80.79, under glasshouse conditions (Table 1). *Vitex trifolia* was found to be natural insecticide in the management of whiteflies. Percent reduction in number of whiteflies over control were recorded as 69.30 (Table 1), under glasshouse conditions. Moreover, the number of plants infected was less in PGPR treated tomato plants compared to untreated control (Table 1).

In the current research T_3 (Pf1+Vpt 10+ Chitin + *Vite*x leaf extract) showed very low iTLCV disease incidence (15.5, 21.9, 19.7 in three field trials) and low number of insect vectors compared to other PGPR treatments, insecticide and untreated control. In all trials, the PF1+VPT 10+ Chitin + *Vite*x leaf extract strain and mixture strains with chitin showed lower symptoms of TLCV and high fruit yield (1.70 kg/Plant) compared to other treatments. (Table 2)

In this current research *Vitex trifolia* leaf extract as foliar spray (2 %) in PGPR treated tomato plants added advantage. It effectively managed both whitefly nymphs and adults population (See Table 2) which in turn, reduced iTLCV incidence and increased fruit yield both under glasshouse and field conditions. Especially with Pf1+Vpt 10+ chitin + *Vite*x leaf extract combinations, performed better.

In the present study, the DAS-ELISA test was performed for the detection of TLCV in PGPR treated tomato plants and the ELISA values were found to be lower in PGPR treated leaf samples especially in Pf1 + VPT10 +chitin + *Vite*x leaf extract compared to the untreated control plants. Apart from these, TLCV was detected in whitefly nymphs and adults collected from PGPR treated tomato plants and untreated control. (Table 3)

Discussions

Both single and strain mixtures of *Pseudomonas* with chitin showed better control of iTLCV and its whitefly vector in tomato. This finding is in line with previous research work of Zehnder *et al*. (2000) [11] and Murphy *et al*. (2000) [11]. Delayed TLCV symptom expression up to nine additional days from the date of challenge inoculation were observed under greenhouse conditions. Moreover, the number of plants infected was less in PGPR treated tomato plants compared to untreated control. These results agree with previous studies of Maurhofer *et al*. (1994) [10], Raupach *et al*. (1996) [17], Murphy *et al*. (2000) [11] and Vasanthi (2001) [25] in the management of tobacco necrosis virus (TNV) in tobacco, cucumber mosaic virus (CMV) in tomato, tomato mottle virus (ToMoV) in tomato and tomato spotted wilt virus (TSWV) in tomato, respectively.

PGPR-mediated ISR has been exploited for the management of virus diseases successfully under field conditions (Zehnder *et al*. 2000) [11]. Earlier reports revealed reduced ToMoV incidence and disease severity in PGPR treatments (Murphy *et a*l. 2000) [11]. Commercial development PGPR+chitosan were formulated and evaluated for plants to manage disease. (Zehnder *et al*, 2000 and Senthilraja *et al*, 2010) [11, 18]. Management of TSWV using different PGPR strains, i.e. individual and mixture strains in tomato under field conditions was reported by Vasanthi (2001) [25] and Kandan *et al*. (2005) [5]. All the above research data for the management of virus diseases supported our findings for the management of iTLCV with PGPR strains. The efficacy of PGPR strains significantly reduced the iTLCV symptoms in tomato under field conditions. Hence strain mixtures contributed additional mechanisms for better management and yield.

Vitex trifolia leaf extract contain phytochemicals viz., alkaloids, saponin, tannin, phenols, terpenoids, flavonoids, steroids which are potentially effective in managing insect adults and larvae as reported by Kannathasan *et al*.(2008) [6]. Vector-borne diseases caused by mosquitoes in tropical and sub-tropical countries are effectively managed by *Vitex trifolia (*Kannathasan, *et al*., 2011) [7]. Bean pod sucking pest *Rapturous linearis* was effectively controlled by three *Vitex* Species such as *Vitex altissima, Vitex negundo* and *Vitex trifolia* (John britto and Steena, 2014) [20]. *V. negundo* possess much potential as a bio pesticide.

The ELISA values were slightly lower in whiteflies obtained from PGPR treatment (Pf1 + VPT10 +chitin) and from the untreated control. But, the whitefly nymphs had more virus titres than the adults. This is in line with the previous findings of Murphy *et al*. (2000) [11], Vasanthi (2001) [25], Kandan *et al.* (2005) [5] and (Vasanthi and Samiyappan, 2017) [22]. They tested the PGPR treated tomato against the ToMoV, TSWV, iTLCV infected samples randomly through dot-blot assay and indirect ELISA and reported less disease symptoms and low virus titre in PGPR treatments than untreated control under field conditions.

In addition to suppression of the disease, the PGPR treatment greatly induced the plant growth. Both under greenhouse and field experiments, the PGPR treated plants showed increased yield of the tomato plants compared to the untreated control plants (Tables 1 and 2). Similar results were reported by Viswanathan and Samiyappan (2002) [27] and Nandakumar *et al*. (2001) [14]. Thus, in our present study, we observed better action of PGPR strains with chitin either singly or in combination along with *Vitex trifolia (*Natural insecticide*)* in managing iTLCV in tomato and the vector population in a ecofriendly way.

Table 1: Effect of PGPR strains, *Vitex* leaf extract and insecticide on the occurrence of disease incidence percentage in glasshouse studies.

S. No	Treatments	Percentage disease incidence	Percent reduction over control	No of whiteflies present	Percent reduction over control
T_1	VPT 10 + Chitin + iTLCV	17.0 ^{c}	67.30	12.5 ^{c}	54.54
T_2	PF1+ Chitin + iTLCV	18.0 ^{c}	65.38	12.0 ^{c}	56.36
T_3	PF1+VPT 10 + Chitin + *Vitex* leaf extract + iTLCV	10.0 ^{e}	80.79	8.5^{d}	69.93
T_4	VPT 10 + Chitin + *Vitex* leaf extract+ iTLCV	14.0 ^{d}	73.03	10.5 ^{d}	61.3
T_5	*Vitex* leaf extract alone + iTLCV	25.0 ^{b}	51.9	14.0 ^{b}	49.9
T_6	Dimethoate + iTLCV	27.0 ^{b}	48.07	16.0 ^{b}	41.8
T_7	Control (untreated) + iTLCV	52.0 ^{a}	0.00	27.5 ^{a}	-
T_8	Healthy control	0.00	-	0.00	-

Each value is the mean of three replicates. Percentage data were arcsine transformed prior to ANOVA. Means followed by same letter do not differ significantly at the 5% probability level by DMRT. Numbers in the parenthesis are arcsine transformed values.

Table 2: Effect of PGPR treatment *Vitex* leaf extract and insecticide on iTLCV in tomato under field conditions.

S. No	Treatments	Trial I	Yield (kg/plant)	Trial II	Yield (kg/plant)	Trial III	Trial III	No of whiteflies	Average Disease Incidence
T_1	VPT 10 + Chitin + iTLCV	22.4 ^{c}	1.40^{d}	32.5^{d}	1.30^{c}	26.4^{c}	1.36^{d}	12.0 ^{c}	27.10^{d}
T_2	PF1+ Chitin + iTLCV	20.6 ^{c}	1.30^{c}	24.0 ^{e}	1.40^{d}	25.3^{c}	1.50^{e}	12.0 ^{c}	23.30^{e}
T_3	PF1+VPT 10+ Chitin + *Vitex* leaf extract + iTLCV	15.5 ^{e}	1.60^{e}	21.9^{f}	1.70^{e}	19.7^{f}	1.63^{f}	9.5 ^{d}	21.60^{f}
T_4	VPT 10 + Chitin + *Vitex* leaf extract	19.3 ^{d}	1.45^{d}	28.0^{d}	1.70^{f}	22.8 ^{d}	1.60^{f}	10.0 ^{d}	23.36^{e}
T_5	*Vitex* leaf extract alone + iTLCV	31.8 ^{b}	1.12^{b}	35.8^{c}	1.27^{b}	33.5^{b}	1.25 ^{c}	15.5 ^{b}	33.70^{c}
T_6	Dimethoate + iTLCV	33.5 ^{b}	1.00^{b}	38.9^{b}	1.10^{b}	37.2 ^{b}	1.04 ^{b}	16.5^{b}	47.13^{b}
T_7	Control (untreated) + iTLCV	49.8 ^{a}	0.76 ^{a}	63.3^{a}	0.90^{a}	57.1^{a}	0.87^{a}	31.0 ^{a}	56.73^{a}
T_8	Healthy control	0.00	1.14^{b}	0.00	1.15^{b}	0.00	1.12^{b}	-	-

Individual and strain mixtures were applied as seed treatment, root dipping, soil application and foliar spray. The data were arcsine transformed before analysis. Data followed by the same letter in a column are not significantly different by DMRT at the 5% level

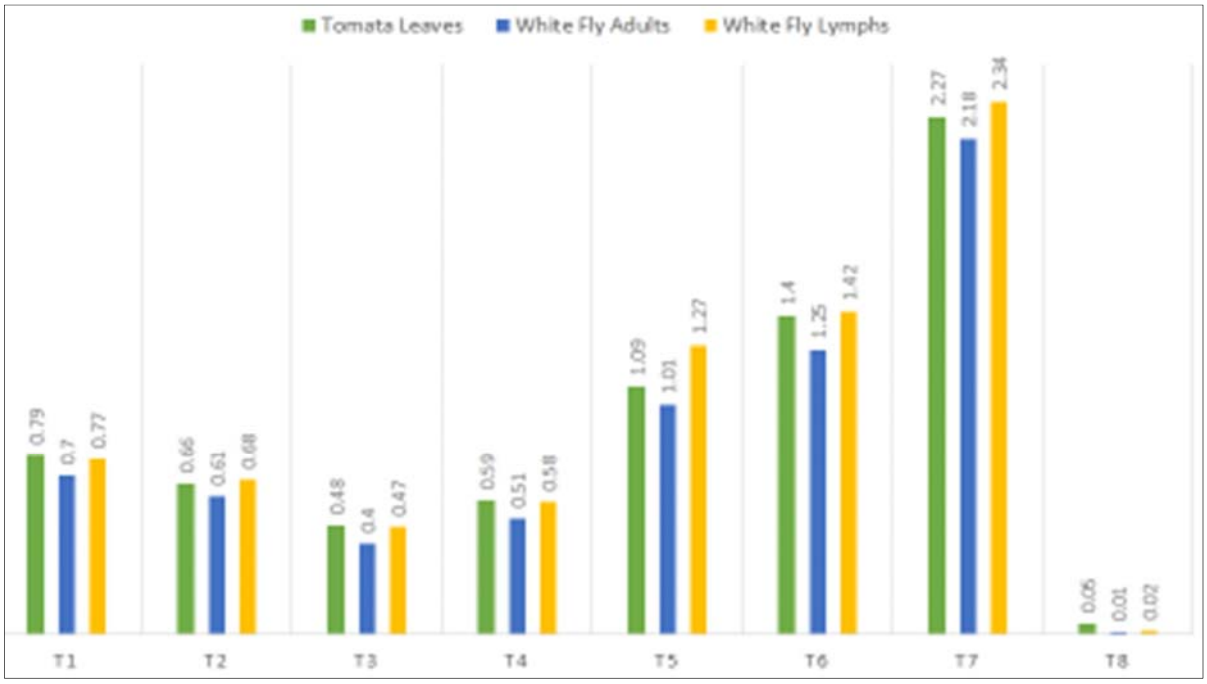

Fig 1: Serological Detection of TLCV in tomato leaves, white fly adults and nymphs under field condition (A 405 nm) $T_{1)}$ Vpt 10 + Chitin + iTLCV. $T_{2)}$ Pf1+ Chitin + iTLCV, $T_{3)}$ Pf1+Vpt 10+ Chitin + *Vitex* leaf extract + iTLCV, $T_{4)}$ Vpt 10 + Chitin + *Vitex* leaf extract, $T_{5)}$ *Vitex* leaf extract alone + iTLCV, $T_{6)}$ Dimethoate + iTLCV, T_7 Control (untreated) + iTLCV. $T_{8)}$ Healthy control.

References

1. Almusa A. Incidence, economic importance and control of tomato yellow leaf curl in Jordan. Plant Dis. 1982; 66:561-563.
2. Behjatnia SAA, Dry IB, Krake LR, Conde BD, Connelly MI, Randles JN, Rezaian MA. New potato spindle tuber viroid and tomato leaf curl geminivirus strains from a wild *Solanum* sp. Phytopathology. 1996; 86:880-886.
3. Gomez KA, Gomez AA. Statistical procedure for agricultural research. New York: John Wiley and Sons, 1984, 320-356.
4. Hossain M. Antibacterial activity of *Vitex trifolia*.

Fitoterapia. 2001; 72(6):695-697.
5. Kandan A, Ramiah M, Vasanthi VJ, Radjacommare R, Nandakumar R, Ramanathan A, *et al.* A novel rhizobacteria based bio-formulation for the management of tomato spotted wilt virus and enhanced yield in tomato. Bio. Cont. Sci Tech. 2005; 15:553-569.
6. Kannathasan K, Senthilkumar A, Venkatesalu V. Larvicidal activity of fatty acid methyl esters of *Vitex* species against *Culex quinquefasciatus*. Parasitol Res. 2008; 103(4):999-1001.
7. Kannathasan K, Senthilkumar A, Venkatesalu V. In vitro antibacterial potential of some *Vitex* species against human pathogenic bacteria. Asian Pac J Trop Med. 2011; 4(8):645-8.
8. King EO, Ward MK, Raney DE. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med. 1954; 44:301-307.
9. Manjunatha BK, Vidya SM, Krishna V, Mankani KL, Singh SDJ, Manohara YN. Comparative evaluation of wound healing potency of Vitextrifolia L. and Vitexaltissima L. Phytotherapy Research. 2007; 21(5):457-461.
10. Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the gac A gene and of pyoverdine production. Phytopathology. 1994; 84:139-146.
11. Murphy JF, Zehnder GW, Schuster DJ, Sikora EJ, Polston JE, Kloepper JW. Plant growth promoting rhizobacterial mediated protection in tomato against tomato mottle virus. Plant Dis. 2000; 84:779-784.
12. Murugan M, Mohan VR. Efficacy of Different Extracts of *Vitex trifolia* L. and *Aristolochia indica* L. for Potential Antibacterial Activity. Science Research Reporter. 2012; 2(1):110-114.
13. Nancy IMR, Meenashree B, Vasanthi VJ. Screening of antibacterial activity and qualitative and quantitative analysis of Phytochemicals of *Vitex trifolia*. Int J Curr Microbiol. App. Sci: I SSN: 2319-7706. 2014; 3(5):425-431.
14. Nandakumar R, Viswanathan R, Babu S, Raguchander T, Samiyappan R. Induction of systemic resistance in rice against sheath blight disease *by Pseudomonas fluorescens*. Soil Biology Biochem. 2001; 33:603-612.
15. Nayar K. Development and evaluation of a biopesticide formulation for control of foliar diseases of rice [Ph.D. thesis]. Coimbatore: Tamil Nadu Agricultural University. Pal BF, Tandon RN. 1937. Types of tobacco leaf curl in Northern India. Indian J Agric Sci. 1996; 7:363-393.
16. Ragupathi N. Studies on leaf curl virus disease of tomato [Ph.D. thesis]. Coimbatore: Tamil Nadu Agricultural University, 1995.
17. Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW. Induced resistance in cucumber and tomato against cucumber mosaic cucumo virus using plant growth promoting rhizobacteria. Plant Dis. 1996; 80:891-894.
18. Senthilraja G, Anand T, Durairaj C, Raguchander T, Samiyappan R. Chitin-based bio formulation of Beauveria bassiana and Pseudomonas fluorescens for improved control of leaf miner and collar rot in groundnut**.** Crop Protection. 2010; 29:1003-1010
19. John DBA, Herin SGD, Benjamin JRKP. Qualitative and quantitative analysis of Phytochemicals in *Marsilea minuta*. Linn. Int J Pharm. Bio. Sci. 2013; 4(1):800-805.
20. John De Britto A, Steena Roshan Sebastian. Screening of bio pesticidal potential of *Vitex* species against *Ripturous linearis* (fabricius). International journal of universal pharmacy and bio sciences, 2014, 194-16.
21. Saikia AK, Muniyappa V. Ultrastructural changes in phloem cells of leaf curl affected tomato from India J Phytopathol. 1989; 124:1-6.
22. Vasanthi VJ, Samiyappan R. Serological detection and diagnosis of indian Tomato leaf curl virus (iTCLV) in tomato and in whitefly nymphs and adults using DAC-ELISA under field conditions**.** National Symposium on Recent Advancements in Agricultural Technologies (NSRAAT), on Gandhigram. Dindigul, Tamil Nadu, India, 2017, 15-16.
23. Vasanthi VJ, Radhajeyalakshmi R, Nasrin F. Anticancer activity of *Vitex trifolia* in two cancer cell lines Int J Phyotochemistry and Pharmacognosy. 2014; 13(7):1013-16.
24. Vasanthi VJ, Kandan A, Raguchander T, Ramanathan A, Balasubramanian P, Samiyappan R. *Pseudomonas fluorescens* -based formulations for management of tomato leaf curl gemini virus (TLCV) and enhanced yield in tomato, Archives of Phytopathology. 2010; 17:553-569.
25. Vasanthi VJ. Management of two major insect-transmitted virus diseases in tomato using plant growth promoting rhizobacteria. Ph.D. Thesis submitted to Tamil Nadu Agricultural University, Coimbatore, 2001, 641003.
26. Vidhyasekaran P, Rabindran R, Muthamilan M, Nayar K, Rajappan K, Subramanian N, *et al.* Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. Plant Pathol. 1997b; 46:291-297.
27. Viswanathan R, Samiyappan R. Induced systemic resistance by fluorescent pseudomonads against red rot disease in sugarcane caused by *Colletotrichum falcatum*. Crop Prot. 2002; 21:1-10.