J. Soils and Crops 31 (2) 190-196, December 2021

ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

MICROBIAL BIOFORMULATIONS FOR MANAGEMENT OF CITRUS CANKER AND REGULATION OF DEFENCE RELATED PLANT CHEMICALS

Swagata Saikia[1], Popy Bora[2] and L.C. Bora[3]

ABSTRACT

Citrus canker caused by *Xanthomonas citri* pv. *citri*(*Xcc*) is one of the most destructive diseases of citrus. We investigated the citrus canker disease management in Experimental Farm of AAU, Jorhat, Assam (2017-19) using three microbial bioformulations *viz.,* Biogreen-5 (Combination of *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichoderma viride*), Bioveer (*Trichoderma viride*) and Biosona (*Beauveria bassiana*) in Assam lemon (*Citrus limon* L. Burm. f) as a test crop, a premier commercial citrus cultivar of Assam. These bioformulations were observed highly effective in suppressing citrus canker incidence and enhancing fruit yield. Biogreen-5 applied as soil application as well as foliar spray showed maximum reduction in canker incidence (35.55 to 20.96%) coupled with an enhanced fruit yield (12.43 to 41.93%). The induction of defence related bio chemicals in citrus plants in response to application of bioformulations was also assayed. Biogreen-5 applied as soil application as well as foliar spray recorded highest phenol, flavanoid, alkaloid and defence enzyme concentration under field condition.

(Key words: Bioformulation, citrus canker, defence enzyme, phenol, flavanoid, alkaloid)

INTRODUCTION

Citrus species probably originated in North-eastern India in Burma and in the adjoining areas. Early in the spread of citrus, some species crossed into China where the sweet oranges, the mandarins and Kumquat developed (Patil *et al.,* 2017). Citrus is the third most important fruit crop in our country after banana and mango (Patil *et al.,* 2017). Citrus canker one of the most feared citrus diseases, affecting all types of important citrus crops. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic conditions (Das, 2003). The pathogen causes necrotic lesions on leaves, stems and fruit. Severe infections can cause defoliation, badly blemished fruit, premature fruit drop, twig dieback and general tree decline (Etebu, 2014). Assam lemon one of the major commercial citrus cultivars of northeast India, including Assam is seen to be most severely affected. For management of citrus canker alot of chemical pesticides (copper sulphate, Bordeaux mixture) and antibiotics (Streptocycline) has been advocated (Das, 2003; Nasreen *et al.,* 2020). But as chemical pesticides are hazardous and degrade environment in an alarming way, therefore, concerned farmers, scientists and conservationists are thinking of sustainable farming in an eco friendly manner. Considering the fact that, the crops had their own natural system of resistance to insect pests and pathogens. At this juncture, an approach in an integrated way to reduce the use of harmful chemicals is of utmost importance. Therefore, a biointensive package needs further strengthening to manage pest and diseases and to meet nutritional requirement of crops, soil health in an eco-friendly way .

Biological control agents, including antagonistic microbes, plant growth-promoting bacteria have proved to be a good option in this regard. Certain strains of plant growth promoting *Pseudomonas* spp. have been already used as biocontrol agents to suppress citrus canker, and offer an attractive way to improve crop growth and development, replacing or supplementing chemical pesticides (Pandeya, 2015). Biopesticides the naturally occurring formulations made from the substances that control pests and diseases by non toxic mechanisms and in ecofriendly manner, are regarded as new technologies. Biopesticides being living organisms (natural enemies) or products there of pose less threat to the environment and to human health, hence can be used for eco-safe management of pests and diseases (Kumar, 2015).

In order to fight infection caused by pathogens or injuries, plants release some secondary compounds like phenolics, flavanoids and alkaloids for its self defence. These secondary metabolites act as protective agents, inhibitors, natural animal toxicants and pesticides against invading organisms, *i.e.* herbivores, nematodes,

1. Ph.D. Scholar, Dept. of Plant Pathology, AAU, Jorhat- 13
2. Jr. Scientist, Dept. of Plant Pathology, AAU, Jorhat- 13
3. Ex. Professor & HOD, Dept. of Plant Pathology, AAU, Jorhat- 13

phytophagous insects and fungal and bacterial pathogens (Lattanzio *et al.*, 2006). Phenolics serve a dual function of both repelling and attracting different organisms in the plants/surroundings. Terpenes and terpenoids, primary constituents of the essential oils of many types of plants and flowers play a major role in plant defense against herbivory, disease resistance, attraction of mutualists such as pollinators, as well as potentially plant-plant communication. Alkaloids are one of the largest groups of plant secondary metabolites, being present in several economically relevant plant families. Alkaloids can act as defense compounds in plants, being efficient against pathogens and predators due to their toxicity. The presence of alkaloids and other secondary metabolites in plants enhances plant reproductive rates, either by improving defenses against biotic and abiotic stresses or by affecting pollinators and seed/fruit disperser visitation. Similarly, plants also have developed a number of mechanisms to cope with stresses that are regulated by multiple signaling pathways (Glazebrook, 2001 and Knight and Knight, 2001), out of that defence response triggered by the enzymes are more critical to resistance (Simmons, 1994; Burns *et al*., 1998). At molecular level, resistance results in an increase in the concentration of metabolites and enzymes related to defensive mechanisms, such as Phenyl alanine ammonia lyase (PAL), Peroxidase (POX), Polyphenol Oxidase (PPO) and â 1,3 glucanase (Stacey *et al.*, 1999). Knowledge of the biochemical and molecular bases of induced resistance is very scanty as only a few studies have been made on enzymes like PAL, POX, PPO and â 1,3 glucanase (Ballester, 2009).

With this background the present study has been attempted to determine the suitability of bio-intensive approach for sustainable management of citrus canker using microbial based bioformulations and assessing the regulation of defence related chemicals in citrus plants.

MATERIALS AND METHODS

The pathogenic bacterium, *Xanthomonas citri* pv. *citri* (*Xcc)* was isolated from diseased citrus leaves (Assam lemon), and was characterized culturally, morphologically and biochemically following standard protocols. The Electron Microscopy of the bacterium was done under a Field Emission Scanning Electron Microscope (FESEM), while molecular characterization was done using 16SrDNA sequencing.

Field evaluation of different bioformulations

The bioformulations were evaluated under field condition for their efficacy in suppression of citrus canker incidence, leaf miner infestation and enhancement of citrus fruit yield. Microbial bioformulations were applied as foliar spray alone or along with soil application. Microbial suspension were prepared with 2 kg of microbial bioformulations (Biosona, Bioveer and Biogreen-5) mixed with 100 l of water and sprayed in the citrus leaves @ 1L plant^{-1}. Moreover, 1kg of these bioformulations was mixed with 100 kg of vermicompost and applied at the base of the plant @ 1 kg plant^{-1}. The spraying of bioformulations was done at an interval of 15 days for 5 months from April'2017 (Total 10 sprays). The experiment was conducted in randomized block design with 7 treatments each with 3 replications. Each replication consisted of 12 lemon plants (Assam Lemon), while record of canker disease, leaf miner incidence and yield assessment were performed after each spray.

Disease incidence

The plants are observed individually and severity percentage are recorded and the numerical rating was given as 0, 1, 3, 5, 7 or 9 shown below. Normally, 50 or 100 leaves were observed and individual rating was given. Per cent Disease Index (PDI) was worked out as per the standard grade chart (Reddy, 1982).

Disease score chart

Grade	Description (% leaf area infected)
0	No signs and symptoms
1	0-10% infection
3	11-15% infection
5	16-25% infection
7	26-50% infection
9	More than 50%

The per cent disease index was worked out as described by Mc Kinney's (1923).

$$PDI = \frac{\text{Sum of numerical rating}}{\text{Total no leaves observed x maximum disease grade}} \times 100$$

Leaf miner incidence

The damaged and undamaged newly emerged leaves plant^{-1} shoot were counted for evaluations of leaf minor damage percentage. The damage per cent was calculated with the following formula:

$$\text{Leaves damage per cent} = \frac{\text{Infested leaves}}{\text{Total leaves}} \times 100$$

Yield assessment

The no fruits from the control and treated plants were counted for evaluations of yield enhancement. The yield enhancement was calculated with the following formula:

$$\text{Yield enhancement} = \frac{\text{No. of fruits in treated plants-No. of fruites in control plants}}{\text{No. of fruits in control plants}} \times 100$$

Estimation of biochemical parameters

Assam lemon leaves were randomly collected from both treated and untreated plants to carry out the biochemical analysis. Assam Lemon leaves were collected before and after application of microbial bioformulations. Total phenol was estimated by Folin-ciocalteu method (Bray and Thorpe, 1954). Total flavonoid content (TFC) of the leaf sample was measured according to the colorimetric assay of Kim, Jeong, and Lee (2003). The total alkaloid content

was estimated by the method of Harborne (1973). The Peroxidase (POX) activity was assayed spectrophotometrically (Hartee, 1955).Enzyme activity was expressed as the change in the absorbance of the reaction mixture min^{-1} g^{-1} on a fresh weight basis (Hammerschmidt *et al*., 1982). Polyphenol oxidase (PPO) activity was determined following Mayer *et al*. (1965). Phenylalanine ammonia lyase (PAL) assay was conducted following Whetten and Sederoff (1992). ß-1,3-glucanase activity was assayed colorimetrically (Pan *et al*., 1991).

RESULTS AND DISCUSSION

Field evaluation of microbe based bioformulation in reduction of citrus canker and leaf miner incidence

The three different bioformulations were applied as soil treatment, foliage sprays and their combined application. The incidence of the disease and leaf miner was recorded higher during early part of April as such first spray was made during 1st week of April, 2017 (7th April, 2017). The remaining sprays were made at an interval of 15 days from the 1st spray. Averages of two observations were recorded after each month (2 sprays $month^{-1}$) and data were used for statistical analysis. All the treatments showed different degree efficacy in controlling the disease and leaf miner incidence and also increased the yield as compared to control (Table 2). The lowest disease (20.96%) and leaf miner (25.08%) incidence was recorded after 6th spray with Biogreen-5 (soil + foliar) *i.e.* after 180 days of the 1st spray. Similarly, highest disease reduction (71.28%) and leaf miner suppression (62.02%) was also recorded after 6th spray with Biogreen-5 (soil + foliar) *i.e.* after 180 days of the 1st spray. Similar results were reported by Galapon *et al.* (2017),where reduction of bacterial blight of rice (BLB) incidence was seen due to application of *Trichoderma harzianum* (5.34%), *Metaharzium anisopliae* (1.06%) and Vesicular Arbuscular Mycorrhiza (0.293%). Similar results were also reported by Samrit *et al.*(2020), where application of *Metarhizium anisopliae* along with botanical extracts controlled Rice stem borer affecting production of rice. Yield of citrus fruits also enhanced after 6th spray with Biogreen-5 (soil + foliar) *i.e.* after 180 days after of 1st spray. Other bioformulations, *i.e.,* Biosona and Bioveer could also be reduced the canker incidence and enhanced fruit yield significantly, although less compared to Biogreen-5. The benefits of biocontrol agents to the plant not only include suppression of pathogens but also yield enhancement of the crops. Pascale *et al.* (2017) reported that foliar spray or drenching of *T. harzianum* as well as their respective major secondary metabolites, harzianic acid (HA) and 6-pentyl-a-pyrone (6PP), increased yield in terms of weight (kg) of grape (63% and 97% respectively), as compared to the untreated control. Bora *et al.* (2021) reported *T. viride* based microbial consortia Biogreen superior to individual formulations against grey blight disease of tea coupled with greater green leaf yield.

Effect of different microbe based bioformulations in defence related biochemical changes in Assam Lemon plants

The data of phenol, flavanoid and alkaloid content of Assam Lemon leaves before and after bio-formulation application are presented in the Table 3,4 and 5.Data of enzymes analysis, *i.e.,* phenyl alanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (POX) and â 1,3 glucanase from 24 hrs to 96 hrs are presented in the Table (6,7,8 and 9) respectively. The study revealed that Phenol concentration was highest in plants treated with bioformulation Biogreen-5 (3.84g) as compared to control (1.03g). Nayam *et al.*(2017) reported that phytohormones treated leaves could show higher level of total phenolic compounds (TPC) in citrus plants. Similar results were seen in the study that revealed Flavanoid content to be highest in plants treated with bioformulation Biogreen-5 (2.25g) as compared to control (1.02g). Daniel *et al.* (2015) reported that application of a plant growth promoting rhizobacterium (PGPR), *Pseudomonas fluorescens*, trigger flavonoid biosynthesis as part of an induced systemic response (ISR) in the blackberry fruit. Alkaloid content was also highest in those plants treated with Biogreen-5 (1.92g) as compared to control (0.19g). Activity peaks of the enzymes, *i.e.* phenyl alanine ammonia lyase (2.425 µmol of transcinnamic acid $min^{-1}g^{-1}$), polyphenol oxidase (1.77 Abs $min^{-1}g^{-1}$), peroxidase (5.25 Abs $min^{-1}g^{-1}$) and â 1,3 glucanase (1.005 µmol of glucose $min^{-1}g^{-1}$) were recorded 72 hrs after application of microbe based bioformulations.Plants treated with Biogreen-5 (Soil and foliar application) showed much higher concentration of enzymes followed by bioformulations Bioveer and Biosona. It was seen that mixture of various biocontrol agents in Biogreen-5 induced more defence enzymes than Bioveer and Biosona. Previous reports demonstrated that mixtures of bacterial strains were found to be more effective in controlling many plant diseases when compared to a single strain as multiple modes of action may be involved (Kavino *et al*., 2008). Up-regulation of enzymatic activities started from 24 hrs and gradually increased in 48 hrs and highest peak was recorded in 72 hrs, after which it declined in 96 hrs. Results found in this study coincides with Wang *et al*. (2010), who reported that *P. fluorescens* applied citrus plants showed peak activities of â-1,3-glucanase (GLU), peroxidase (POX), and phenylalanine ammonia lyase (PAL) effective against green mould on postharvest disease of citrus. The POX activity of treatment with *P. fluorescens* increased sharply in the initial 2 days and then decreased gradually and was lower than the control after the fourth day. The POX activity of citrus treated with *P. fluorescens* was 21.2 per cent higher than the control, while reaching the peak at the forth day with the level of 4.35 U mg^{-1}. Hasabi *et al.* (2014) reported that citrus plants treated with L-arginine, L-methionine, L-ornithine, and distilled water showed changes in â-1,3-glucanase transcript levels and activity of antioxidant enzymes, catalase, peroxidase, and PAL. Based on the results of phenotypic activity, antioxidant enzyme activity and a molecular study of the stressed plants, it was found that activity of â-1, 3-

Table 1. Efficacy of different microbe based bioformulations on canker disease and leaf miner incidence (%)

Treatments	Disease and leaf miner incidence (%) after 12 sprays of different bioformulations at 15 days interval	
	Disease incidence (%)	Leaf miner incidence (%)
Biogreen (Soil & Foliar Application)	20.96 (27.27)	25.08 (30.07)
Biogreen (Foliar Application)	20.14 (28.11)	26.09 (30.72)
Bioveer (Soil & Foliar Application)	43.54 (41.32)	40.05 (39.58)
Bioveer (Foliar Application)	46.39 (42.94)	41.45 (40.11)
Biosona (Soil & Foliar Application)	58.51 (49.89)	56.10 (48.50)
Biosona (Foliar Application)	57.62 (49.37)	55.23 (47.98)
Control	72.99 (57.42)	70.35 (57.04)
S Ed (±)	1.90	1.44
C $D_{(P=0.05)}$	4.08	3.09

*Data in the parenthesis are angular transformed values

Table 2. Efficacy of different microbe based bioformulations on canker disease reduction (%), leaf miner suppression (%) and yield enhancement

Treatments	Disease reduction of citrus canker(%), leaf miner suppression (%) and fruit yield enhancement(%) after 12 sprays of different bioformulations at 15 days interval		
	Disease reduction (%)	Leaf miner suppression (%)	Yield enhancement (%)
Biogreen (Soil & Foliar Application)	71.28 (57.61)	62.02 (52.00)	41.93 (40.40)
Biogreen (Foliar Application)	72.40 (58.31)	62.91 (52.54)	34.78 (36.15)
Bioveer (Soil & Foliar Application)	40.34 (39.47)	43.07 (41.03)	31.29 (34.02)
Bioveer (Foliar Application)	36.44 (37.17)	41.08 (39.87)	24.36 (29.60)
Biosona (Soil & Foliar Application)	19.83 (26.49)	20.25 (26.78)	23.07 (28.73)
Biosona (Foliar Application)	21.05 (27.35)	21.49 (27.62)	17.43 (24.73)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S Ed (±)	2.28	2.08	1.24
C $D_{(P=0.05)}$	4.56	5.25	2 .44

*Data in the parenthesis are angular transformed values

Table 3. Effect of different microbe based bioformulations on phenol content (g) of citrus leaves

Treatments	Before application	After application
T1=Biogreen (Soil and foliar application)	2.89	3.84
T2=Biogreen (Foliar Application)	2.45	3.52
T3=Bioveer (Soil and Foliar Application)	2.87	2.38
T4=Bioveer (Foliar Application)	2.56	2.07
T5=Biosona (Soil and Foliar Application)	2.21	2.09
T6=Biosona (Foliar Application)	1.78	1.89
T7=Control	2.00	1.03

Table 4. Effect of different microbe based bioformulations on flavanoid content (g) of citrus leaves

Treatments	Before application	After application
T1=Biogreen (Soil and foliar application)	1.67	2.25
T2=Biogreen (Foliar Application)	1.50	2.09
T3=Bioveer (Soil and Foliar Application)	1.52	2.00
T4=Bioveer (Foliar Application)	1.21	1.69
T5=Biosona (Soil and Foliar Application)	1.15	1.53
T6=Biosona (Foliar Application)	1.09	1.27
T7=Control	1.00	1.02

Table 5. Effect of different microbe based bioformulations on alkaloid content (g) of citrus leaves

Treatments	Before application	After application
T1=Biogreen (Soil and foliar application)	0.09	1.92
T2=Biogreen (Foliar Application)	0.07	1.90
T3=Bioveer (Soil and Foliar Application)	0.05	1.45
T4=Bioveer (Foliar Application)	1.00	1.76
T5=Biosona (Soil and Foliar Application)	0.39	0.86
T6=Biosona (Foliar Application)	0.19	0.90
T7=Control	0.01	0.19

Table 6. Activity of PAL (µg of transcinnamic acid $min^{-1}g^{-1}$) in citrus leaves after application of microbe based bioformulations

Treatments	Time interval				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T1=Biogreen (Soil and Foliar Application)	2.167	2.209	2.293	2.425	2.064
T2=Biogreen (Foliar Application)	2.067	2.000	2.208	2.345	2.085
T3=Bioveer (Soil and Foliar Application)	0.722	0.850	0.482	0.878	0.508
T4=Bioveer (Foliar Application)	0.905	0.967	1.000	1.005	0.943
T5=Biosona (Soil and Foliar Application)	0.930	0.956	0.809	1.783	0.850
T6=Biosona (Foliar Application)	0.912	0.987	1.006	1.064	0.994
T7=Control	0.607	0.722	0.853	0.878	0.912

Table 7. Activity of PPO (Abs $min^{-1}g^{-1}$) in citrus leaves after application of microbe based bioformulations

Treatments	Time interval				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T1=Biogreen (Soil and Foliar Application)	0.770	0.990	1.000	1.770	0.900
T2=Biogreen (Foliar Application)	0.750	0.950	0.990	1.110	0.870
T3=Bioveer (Soil and Foliar Application)	0.006	0.005	0.200	0.320	0.089
T4=Bioveer (Foliar Application)	0.067	0.100	0.012	0.370	0.056
T5=Biosona (Soil and Foliar Application)	0.145	0.128	0.189	0.270	0.006
T6=Biosona (Foliar Application)	0.007	0.090	0.008	0.200	0.101
T7=Control	0.012	0.023	0.005	0.060	0.156

Table 8. Activity of POX (Abs $min^{-1}g^{-1}$) in citrus leaves (Assam lemon) after application of microbe based bioformulations

Treatments	Time interval				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T1=Biogreen (Soil and Foliar Application)	3.080	3.910	4.080	5.250	2.660
T2=Biogreen (Foliar Application)	3.070	3.090	4.010	4.560	1.090
T3=Bioveer (Soil and Foliar Application)	1.160	1.050	2.660	2.090	1.250
T4=Bioveer (Foliar Application)	1.080	1.330	1.500	1.750	0.250
T5=Biosona (Soil and Foliar Application)	1.030	1.130	2.010	1.440	0.760
T6=Biosona (Foliar Application)	0.540	0.740	0.800	0.910	0.840
T7=Control	0.061	0.067	0.008	1.001	0.009

Table 9. Activity of β-1, 3- glucanase activity (µg of glucose $min^{-1}g^{-1}$) of citrus leaves after application of microbe based biofomulations

Treatments	Time interval				
	0 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T1=Biogreen (Soil and Foliar Application)	0.072	0.090	0.060	1.005	0.091
T2=Biogreen (Foliar Application)	0.093	0.098	0.090	1.001	0.090
T3=Bioveer (Soil and Foliar Application)	0.201	0.100	0.228	0.210	0.990
T4=Bioveer (Foliar Application)	0.320	0.534	0.690	0.670	0.578
T5=Biosona (Soil and Foliar Application)	0.250	0.290	0.390	0.310	0.300
T6=Biosona (Foliar Application)	0.210	0.490	0.548	0.450	0.320
T7=Control	0.060	0.062	0.064	0.060	0.061

glucanase, peroxidase and PAL enzyme activity was significantly induced at 48 and 72 h after inoculation, but especially 72 h after inoculation.

Our results showed that there was a significant decrease in citrus canker disease incidence along with increase in the level of biochemical constituents when the crop was treated with different microbial bioformulations. The results depicted that major diseases could be controlled up to a significant level by upregulation of defence mechanisms of plants. As canker proves to be a prominent hindrance in its productivity and qualitative yield, emphasis needs to be given to manage it with some alternative means like biointensive means reducing the chemical load on the crop.

RERERENCES

Ballester, A., D. Batabyala, T. Egawwa, C. Lua, Y. Lina, A. Marcelo, C. Martib, L. Capeceb, A. Dario and S. Yeha, 2009. Evidence for a ferryl intermediate in a heme-based dioxygenase. Proc. Natl. Acad. Sci. **106** (41) : 17371-17376

Bray, H. and W. Thorpe, 1954. Analysis of phenolic compounds of interest in metabolism.Meth.Biochem.Anal.**1**:27-52.

Bora, P., L. C. Bora and R.P. Bhuyan, 2021. Evaluation of botanicals and microbial bioformulations for management of grey blight disease of tea under organic production system. *Indian J. Agri. Sci.*. **91** (1): 54–57.

Burns, J., D. Lewandowski, C. Nairn and G. Brown, 1998. Endo1,4-glucanase gene expression and cell wall hydrolase activities during abscission in Valencia orange. Physiol. Plant. **102**: 217-225.

Daniel, G., Z. Yang, J. Francisco, Gutierrez, M. Cathie and R. Beatriz, 2015. Application of *Pseudomonas fluorescens* to Blackberry under Field Conditions Improves Fruit Quality by Modifying Flavonoid Metabolism.Plos One.**23**:1-23.

Das, A. 2003. Citrus canker – A review. J. Appl. Hort. **5**(1):52-60.

Etebu, E. and A. Nwauzoma, 2014. A review on sweet orange (*Citrus sinensis* Osbeck): health, diseases, and management. Am. J. Res. Commun. **2**(2): 33-70.

Galapon, J. and A. Rebong, 2017. Utilizing Plant-Microbial Interactions in Controlling Rice Major Diseases and Increasing Rice Yields. Int. J. agric. Technol. **13**(7.2):1597-1620.

Glazebrook, J. 2001. Genes controlling expression of the defence responses in Arabidopsis. Plant Biol. **4**:301-308.

Hammerschmidt, R., E. Nuckles and J. Kuc, 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colleotrichum lagenarium*. Physiol. Plant Pathol.**20**:73–82.

Harborne, J. 1973. Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London.

Hartree, E. 1955."Haematin compounds". In Modern methods of plant analysis.pp.197- 245.

Hasabi, V., H. Askari, S. Alavi and H. Zamanizadeh, 2014. Effect of amino acid application on induced resistance against citrus canker disease in lime plants. J. Plant Prot. Res. **54** (2):144-149.

Kavino, M., S. Harish, N. Kumar, D. Saravankumar and R. Samiyappan, 2008. Induction of systemic resistance in Banana (Musa spp.) against Banana bunchy top virus (BBTV) by combining chitin with root colonizing *Pseudomonas flourescens* strain CHAO. Eur. J. Plant Pathol.**120**:353-364.

Kim, D., S. Jeong and C. Lee, 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. **81**:321– 326.

Knight, H. and M. Knight, 2001. Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci. **6**:262–267.

Kumar, S. and S. Archana, 2015.Biopesticides: Present Status and the Future Prospects. J FertilPestic.**6**(2):1-2.

Lattanzio, V., V. Lattanzio and A. Cardinalis, 2006.Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects.Phytochemistry: J. Adv. Res. **2006**: 23-67.

Mayer, A., E. Harel and R. Ben-Shaul, 1965.Assay of catechol oxidase—a critical comparison of methods. Phytochemistry.**5**(4):783-789.

Mckinney, H. 1923. A new system of grading of plant diseases. J. Agric. Res.**26**: 195–218.

Nasreen, R., P. Bora and K K Medhi, 2020. Citrus Canker: Developments down the lane. Annals Plant and Soil Res. **22** (4), 396-404.

Nayem, S., M. Salahuddin, M. Chowdhury, F. Aminuzzaman, B. Hossain and S. Ali, 2017.Phytohormone-Induced Resistance against *Xanthomonas axonopodi* spv.*citri* in *Citrus aurantifolia.*Am. J. Plant Sci. **8**:1135-1147.

Pan, S., S. Ye and J. Kurt, 1991. Association of â-1,3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. Physiol. Mol. Plant Pathol. **39**:25-39.

Pandeya, A., B. Pokhrel, R. Sagar and S. Raut, 2015. Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. Afr. J. Biotechnol. **14**(46):3159-3163.

Pascale, A., F. Vinale, G. Manganiello, M. Nigro, S. Lanzuise, M. Ruocco, R. Marra, N. Lombardi, S. Woo and M. Lorito, 2006. Trichoderma and its secondary metabolites improve yield and quality of grapes. Crop Protection.**92**: 176-181.

Patil, S., V. Parthiban, G. Sekar, and K. Marimuthu, 2017. Survey, Isolation and Identification of Post Harvest *Penicillium* mould of Sweet Orange. J. Soils and Crops. **27**(1):45-49.

Reddy, R. 1982. 'Evaluation of fungicides against major diseases of chilli'.Unpublished M.Sc. thesis, Tamil Nadu Agriculture University, Coimbatore, India.

Samrit, R., B. Chaudhari, G. Shamkuwar, P. Neharkar and H. Sawai, 2020. Evaluation of Biopesticide, Botanicals and Plant Extracts against Rice Stem Borer, *Scripophaga incertulas* (Walker). J. Soils and Crops. **30**(1): 124-128.

Simmons, C. 1994. The physiology and molecular biology of plant 1,3—d-glucanase and 1,3;1,4-d-glucanase. Plant Sci. **13**:325–387.

Stacey, R., T. Nordeng, F. Culianez-Macia and R. Aalen, 1999. The dormancy-related peroxiredoxin anti-oxidant, PER1, is localized to the nucleus of barley embryo and aleurone cells. Plant J. **19**:1-8.

Wang, Z., M. Jiang, K. Chen, K. Wang, M. Du, Z. Zala, F. Hegyi and J. Kan, 2010.Biocontrol of *Penicillium digitatum* on Postharvest Citrus Fruits by *Pseudomonas fluorescens*. J. Food Qual. **2018**:1-10.

Whetten, R. and R. Sederoff, 1992. Phenyl alanine Ammonia Lyase from Loblolly Pine.Purification of the Enzyme and Isolation of Complimentary DNA clones. Plant Physiol. **98**:380-386.

Rec. on 20.05.2021 & Acc. on 01.06.2021