Mysore J. Agric. Sci., 51 (3) : 621-624, 2017

In vitro Evaluation of Bioagents against *Fusarium solani* (Mart.) Sacc. Causing Wilt of Betelvine (*Piper betel* L.)

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ABSTRACT

The fungal biocontrol agent *Trichoderma harzianum* (Th-55) and *T. viride* (Tv-21) inhibited 85.00 and 80.56 per cent growth of the pathogen, respectively. Least inhibition was observed with *T. harzianum* strains Th-2 (67.78%) and Th-41 (67.78%). The bacterial bioagents *Bacillus subtilis* (P-24) and *B. subtilis* var. *amyloliquefaciens* (FZB24) inhibited 72.59 and 67.41 per cent growth of the pathogen, respectively. The antagonist *T. harzianum* (Th-55), *T. viride* (Tv-21) and *B. subtilis* (P-24) can be exploit in field conditions for the management of betelvine wilt.

Keywords : Bioagents, wilt, betelvine

BETELVINE is a perennial, dioecious, evergreen climber that is grown in tropics and subtropics for its leaves that are used as a chewing stimulant. The deep green heart shaped leaves of betel vine are popularly known as Paan in India. Betelvine is (*Piper betel* Linn.) is belongs to the family of piperaceae *i.e.,* the black pepper family (Gunther, 1952). In India, betel vine is grown as an important cash crop in southern parts, mainly in the states of Andhra Pradesh, Karnataka, Kerala and Tamil Nadu. Betelvine is cultivated on about 55000 h. with an annual production worth of about Rs. 9000 million. These leaves are also in great demand in several other countries of the world. Betelvine succumb to several fungal diseases damage to crops. However, the most important disease is wilt. The cultivation has been abandoned by many of betelvine growers due to heavy infection with wilt pathogen *F. solani* and the area under cultivation is diminishing steadily. The losses at different locations, which ranged from 4 to 100 per cent due to wilt disease (Saksena, 1977).

Although currently a variety of techniques and methods have been known to manage betelvine wilt disease. The use of chemical based fungicides is most effective and reliable method to control the pathogens. However, fungicides are highly toxic to targeted pathogen, plants, products, human beings and other forms of life. Biological control based antagonists is the popular alternative to chemical control and in most of the cases confined to experimental research. The bio-control agents of some plant pathogens have been found effective and gaining importance as an alternate to chemical control method.

Biological control is the best alternative method for the management soil borne pathogens, compared to chemical control. Among the various antagonists used for the management of plant diseases, *Trichoderma* spp. plays a vital role. Among the various species of *T. asperellum*, *T. harzianum*, *T. virens*, *T. viride* and *T. hamatum* are used for the management of diseases of crop plants against soil bornepathogens. These filamentous fungi are very common innature, with high population densities in soil and plantlitters (Samuels, 1996). Teleomorphs of *Trichoderma* are species of the ascomycetes of genus Hypocrea. Many studies have been proved the potential of *Trichoderma* spp. asbiological agent antagonistic to several plant pathogens (Naseby *et al.,* 2000; Tondje *et al.,* 2007; Houssien *et al.,* 2010). Therefore, the objective of the study was to evaluate different bioagents under laboratory conditions to find out the most effective one for final use. The results of these studies will be helpful to the growers to adopt the most suitable management strategy against *F. solani*.

MATERIAL AND METHODS

The efficacy of twenty fungal and nine bacterial antagonists were evaluated against *F. solani* on the potato dextrose agar medium by using dualculture technique under *in vitro* condition. Observations on

mycelial growth of fungus was recorded seven days of incubation under laboratory condition. Each treatment was replicated three times. Fifteen mililiter of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of *F. solani* was inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. With 70 mm distance between pathogen and antagonist was maintained. In case of evaluation of bacterial antagonist, the test fungus placed at the center of the Petri plate one day earlier and bacterium was streaked around test fungus. The plates were incubated at 27 ± 1 ºC and zone of inhibition was recorded by measuring the inhibition of the test fungus by the antagonistic organism. The colony diameter of pathogen in control plate was recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = per cent inhibition
C = growth in control
T = growth in treatment

Results and Discussion

The effect of fungal biocontrol agents was studied *in vitro* against *F. solani*. Three fungal bioagents *viz., T. viride*, *T.harzianum* were used to know their antagonistic potential against *F. solani*. Results revealed that, the effect of bioagents on fungal growth ranged from 67.78 to 85.00 per cent at 7 days after inoculation. Among the fungal antagonists tested, inhibition of radial growth was maximum in case of *T. harzianum* (Th-55) (85.00%), followed by *T. viride* (Tv-21) [80.56 %], *T. harzianum* (Th-22) [79.81%], *Trichoderma* (T-B2) [79.26 %] and *T. harzianum* (Th-2 and Th-41) [67.78%] which recorded least inhibition of the radialgrowth of the pathogen. However, the fungal biocontrol agents were significantly superior in inhibiting the pathogen growth under *in-vitro* condition (Table I and Plate 1).

Similar results were observed in root rot of bean caused by *Fusarium solanif* sp. *phaseoli*.

Table I

In-vitro evaluation of fungal bioagents against F. solani

Fungal bioagents	Per cent Inhibition over control
Trichoderma viride (Tv -13)	75.19 (60.15) *
Trichoderma viride (Tv-16)	78.89 (62.73)
Trichoderma viride (Tv-19)	76.85 (61.26)
Trichoderma viride (Tv-21)	80.56 (63.83)
Trichoderma viride (Tv-NBAIR)	77.96 (62.00)
Trichoderma viride (Tv-22)	76.30 (60.88)
Trichoderma viride (Tv-52)	72.59 (58.52)
Trichoderma viride (Tv-60)	73.89 (59.36)
Trichoderma harzianum (Th-2)	67.78 (55.41)
Trichoderma harzianum (Th-19)	75.56 (60.46)
Trichoderma harzianum (Th-20)	67.96 (55.53)
Trichoderma harzianum (Th-22)	79.81 (63.31)
Trichoderma harzianum (Th -41)	67.78 (55.41)
Trichoderma harzianum (Th-55)	85.00 (67.21)
Trichoderma harzianum (Th-NBAIR)	71.48 (57.72)
Trichoderma harzianum (Th-58)	75.37 (60.26)
Trichoderma (T-B1)	75.37 (60.46)
Trichoderma (T- B2)	79.26 (63.00)
Trichoderma viride	72.96 (58.85)
Trichoderma	71.11 (57.48)
Mean	60.19
SEm±	1.61
CD (P 0.01)	6.18
CV%	4.65

* The values in the parenthesis are arc sine transformed

T. harzianum hyphae coiled around hyphae of *F. solani* resulting vacuolization and disintegration of pathogen hyphae indicating strong antagonistic activity of the *T. harzianum* isolate RU01(Saman Abeysinghe, 2007). Morsy *et al*. (2009) observed efficiency of *T. viride* against *F. solani* on tomato plants. *T. viride* inhibited the over growth of *F. solani* compared to the control. The inhibitionby the strains was attributed

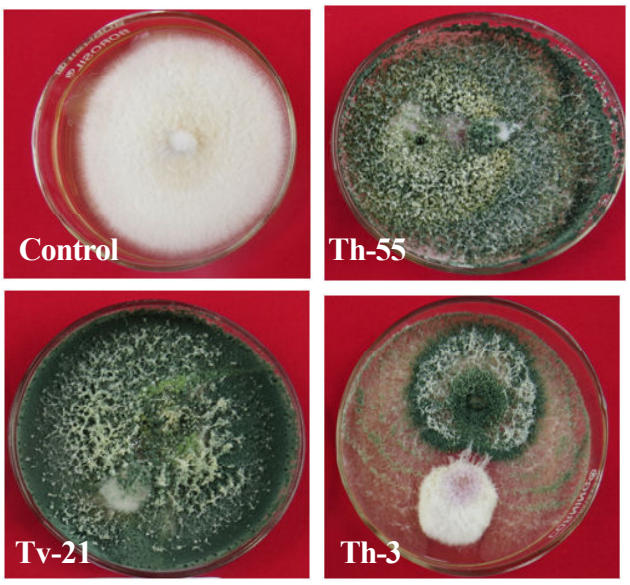

Plate 1: *In vitro* evaluation fungal antagonists against *F. solani.*

to their ability to secrete hydrolytic enzymes or antifungal metabolites. These findings are in harmony with those obtained by Montealegre *et al.* (2005) who reported that *Trichoderma* spp. secreted chitinase and B 1, 3 glucanase in supernatants.

The bacterial biocontrol agents *B. subtilis, Pseudomonas fluorescens* and *B. s.* var. *amyloliquefaciens* were screened against *F. solani*. The effect bacterial antagonists on the fungal growth ranged from 18.67to 72.59 per cent at 7 days after inoculation. Among the bacterial antagonists tested, inhibition of radial growth was maximum in case of *B. subtilis* (P-24) (72.59%), followed by *B. s.* var. *amyloliquefaciens* (FZB24) [67.41 %], *P. fluorescens* (P-4) [58. 67 %] and *B. subtilis* [55.37 %] while *B. subtilis (ME)*[18.67%] was least effective in inhibiting the radialgrowth of the pathogen. However, bacterial antagonists recorded significantly effective in inhibiting the pathogen growth under *in-vitro* condition (TableII and Plate 2).

Similar results were recorded in *Fusarium* wilt of tomato whereas *B. amyloliquefaciens* inhibited the growth of *F. solani* by 95.20 per cent (Caroline Fadeke Ajilogba *et al*., 2013). Also it has been reported that *B. amyloliquefaciens* strains have been able to inhibit the growth of a variety of fungal pathogens because of their ability to produce a vast array of antibiotics such as zwittermicin, bacillomycin, fengycin, bacilysin

TABLE II

In-vitro evaluation of bacterial bioagents against F. solani.

Fungal bioagents	Per cent Inhibition over control	
Bacillus subtilis (ME)	18.67 (25.57)	*
Psuedomonas putida	36.51 (37.21)	
Bacillus subtilis var. *amyloliquefaciens*	67.41 (55.21)	
Bacillus subtilis (P-21)	38.15 (38.13)	
Bacillus subtilis (P-48)	50.37 (45.21)	
Pseudomonas fluorescens (P-4)	58.67 (49.99)	
Psuedomonas (P-37)	22.04 (27.98)	
Bacillus subtilis (P-24)	72.59 (58.50)	
Bacillus subtilis	55.37 (48.08)	
Mean	60.19	
SEm±	1.61	
CD (P 0.01)	6.18	
CV %	4.65	

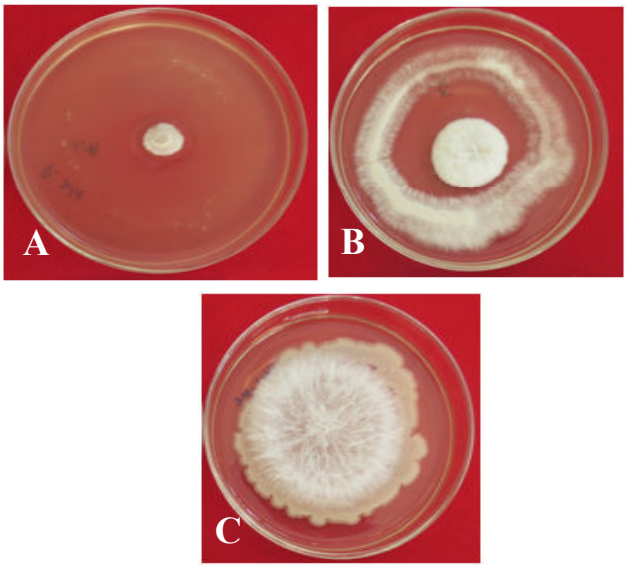

Plate 2*: In vitro* evaluation bacterial antagonists against against *F.solani*. (A*: Bacillus subtilis* (P-24*)* B: *Bacillus subtilis* var. *amyloliquifacians* C: *Bacillus subtilis* (ME))

and difficidin (Chen *et al.*, 2009). Montealegre *et al.* (2005) pointed that the cell free culture filtrate of *B. subtilis* inhibited the mycelial growth, radial growth, spore germination and germ-tubes length of *F. oxysporum*. Moreover, Alippi and Monaco (1994)

reported that *B. subtilis* can secrete several antifungal metabolites such as subtilin, bacitracin, bacillin and bacillomycin which have an inhibitory effect on fungal pathogens.

It can be concluded from the above results that the *T. harzianum* (Th-55), *T. viride* (Tv-21) and *B. subtilis* (P-24) were suppressed growth of betelvine wilt pathogen *F. solani,* and these antagonists can be exploit for the management of betelvine wilt under field condition and save the crop.

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(*Received :* May, 2017 *Accepted :* August, 2017)

Mysore J. Agric. Sci., 51 (3) : 625-630, 2017

Non-chemical Management of Papaya Ring Spot Virus in Papaya (*Carica papaya* L.)

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Abstract

The efficacy of different treatments comprising of different cultural and biomolecules against Papaya ringspot virus (PRSV) was tested under field condition. Growing African tall maize as live barrier with silver reflective mulchand soil and foliar application of micronutrients with neem leaf extract (5ml / litre), neem oil (5ml / litre), Kappaphycus alvarezii-1 (4.0 ml / lt), Kappaphycus alvarezii-2 (6.0 ml / lt) and Synthetic Oligo (0.25mg / litre) was found significantly effective in reducing PRSV incidence (86.76%) compared to border crop of African tall maize alone which as record 73.33 per cent disease control and an average yield of 46.23 kg / plant. Among the bio -molecules, Kappaphycusalvarezii-1has recorded 71.66 per cent disease control with the yield of 41.87 kg / plant. Use of reflective mulch recorded 65.67 per cent disease control with an average yield of 41.57 kg / plant. Spraying with botanicals and biopesticides *viz.,*neemleaf extract and neemoil have recorded 53.33 and 60.00 per cent, respectively. Whereas, untreated control plants showed 100 per cent disease incidence of PRSV.

Keywords: Papaya, papaya ringspot virus, disease control

Papaya (*Carica papaya* L.), is considered as an important fruit crop because of its great economic potential. It belongs to the family Caricaceae and species related to *C. papaya* are *C. pentagona*, *C. cauliflora*, *C. pubescens* and *C. stipulate.* Fruits are low in calories and rich in natural vitamins and minerals. Papaya placed first among the fruits for vitamin-C, A, riboflavin, calcium, thiamine, iron, niacin, potassium and fiber. Hundred gram of ripe fruit contain 0.6 g of protein, 0.1 g of fat, 0.5 g of minerals, 0.8 g of fiber, 7.2 g of carbohydrates, 32 kcal of energy, total carotene of 2,740µm and β-carotene of 888µm (Krishna *et al*., 2008).

Unripe green papaya is also used as vegetable, it contain all other nutrients except carotene. It is also used in salads, pies, sherbets, juices and confections. The fruit is a rich source of latex which contains proteolytic enzymes, papain and chymopapain. Papain is an enzyme that breaks protein and has been commonly used in food, pharmaceutical and cosmetic industries for producing clean singlotions, facial creams and toothpastes. Chymopapain and antioxidant nutrients found in papaya have been found helpful in lowering inflammation and healing burns (Aravind *et al*., 2013).

Indian domestic demand for papaya has become strong because of sizable population, significant rise in per-capita income and growing interest for healthier food products (FAOSTAT, 2012). The congenial climatic conditions in India have wider scope for commercial cultivation and export of papaya. The area and production were in increasing trend during late 20th century, but with increased severity of PRSV during the early 21st century it became a major hurdle for growers and a challenge for scientists leading to a heavy toll of the crop. The virus infects plants of all the age groups, early infection gradually lead to complete loss of yield and later infections reduce the yield and quality of fruit. Symptoms such as mild mosaic, mosaic, puckering, mottling, vein clearing, vein banding, blistering, distortion and shoe strings in severe case appear on leaves after two to three weeks of infection (Plate 1). Oily streaks on petioles, ringspots on leaves and fruits can be observed. In advanced case infected plants appear bushy, back headed, tapering and finally death can also be

Plate 1: Papaya ringspot virus infected papaya plant

noticed. Infected plants flower meagerly and produce malformed fruits with poor taste, market value and keeping quality (Gonsalves, 1994).

The incidence of PRSV is becoming a major limiting factor and is challenging to papaya growers worldwide. Papaya ringspot virus cause heavy loss of 85-90 per cent depending upon the time of infection age of the plant (Usharani *et al*., 2013). Currently reported management methods like cultural, chemical and biological methods for control of the disease, they are ineffective practically. Keeping in view of the limited work on biological approaches, the different management practices was undertaken.

Material and Methods

In order to evaluate the efficiency of integrated management approach to combat PRSV disease, field experiments were laid in farmer field at Byadarahalli village, Devanahalli taluk of Bengaluru rural district of Karnataka and MRS, Hebbal, Bengaluru during 2015-17. The papaya seedlings of a popular variety *viz.,* Red Lady were raised in 6''x 4''polyethylene covers and maintained in insect proof nylon mesh of 40 x gauge. Forty fivedays old seedlings were then transplanted in the main field by maintaining a spacing of 6'x6'. The recommended packageof practices were followed till the end of experiment. Eleven different treatments were imposed and evaluated by using a simple RCBD design with three replications. The observations on plant height, disease incidence, number and weight of fruits were recorded.

Treatments imposed were as follows

T-1: Growing African tall maize as live barrier: Two months before transplanting the papaya seedlings, "African tall maize" was grown densely all around the treatment plot and also in between the rows of papaya as live a barrier in the ratio of 1:1.

T-2: Growing papaya with silver reflective mulch: Forty five days old Red Lady seedlings were grownin row covered with silver reflective mulch which was spread above each papaya row to repel the aphids.

T-3: Soil and foliar application of micronutrients: Micronutrients like Zinc, Boron, Iron and Manganese were applied at monthly intervals.

Micronutrient	Soil application dose	Foliar spray dose
Zinc	10 kg/ha	0.5%
Boron	10 kg/ha	0.5%
Iron	15 kg/ha	1.0%
Manganese	04 kg/ha	0.5%

T-4: Foliar application of micronutrients: Micronutrients like Zinc, Boron, Iron and Manganese were applied at monthly intervals.

T-5: Spray with neem leaf extract @ 5ml /litre: Preparation of neem leaf extract: The neem leaves were covered with water at a ratio of one kilogram of leaves to five liters of water and kept for overnight. The next day leaves were grounded and supernatant was collected and used for spraying.

T-6: Spraying with neem oil @ 5ml / litre: Neem oil @ 5ml / litre was sprayed at monthly intervals.

T-7: Spraying with *Kappaphycusalvarezii-1*: Sea weed *Kappaphycusalvarezii*-1(LBD) was sprayed @ 4ml / litre at monthly intervals.

T-8: Spraying with *Kappaphycus alvarezii*-2: Sea weed *Kappaphycus alvarezii*-2 (LBS) @ 6ml/ litrewas sprayed at monthly intervals.

T-9: Spraying with Synthetic Oligo(SEVI)@ 0.25mg/ litre:SEVI @ 0.25mg/litre was sprayed at monthly intervals.

T-10:Combination of all treatments:All the above mentioned treatments were combined.

T-11:Untreated control: Forty fivedays old papaya seedlings of "Red lady" were planted in the main field. These plants were maintained untreated withoutimposing any of the above treatments.

Calculation of Per cent disease incidence

Per cent disease incidence was calculated by recording number of plants infected and total number of plants in a plot.

$$DI\ (\%) = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

The per cent disease reduction over control was calculated by using the formula given by Vincent (1947).

$$\text{Per cent disease reduction} = \frac{(C—T)}{C} \times 100$$

Where,

C = Per cent disease in control

T = Per cent disease in treatment

Statistical analysis : The field experimental data was analyzed statistically by Fischer's method of analysis of variance as given by Panse and Sukhatme (1967). The level of significance used in 'F' test was P=0.01. Critical difference was worked out wherever 'F' test was significant.

Results and Discussion

The popular variety of papaya *viz*., Red Lady was selected to test efficacy of different treatments under field condition against Papaya ringspot virus. Significant difference in disease control, plant height and yield was observed in all the treatments compared with untreated control. Among the different treatments, T_{10} (Growing African tall maize as live barrier with silver reflective mulch and soil and foliar application of micronutrients with neem leaf extract (5ml /litre), neem oil (5ml / litre), *Kappaphycus alvarezii*-1 (4.0 ml / lt), *Kappaphycus alvarezii*-2 (6.0 ml / lt) and Synthetic Oligo (0.25mg / litre) has found significantly efficient in reducing PRSV with 86.76 per cent disease control (Table I and Plate 2)with an average plant height and yield of 4.40 feet (Fig. 2) and 48.52 kg per plant respectively (Table II).

Growing papaya with African Tall maize as live barrier alone (T1) has recorded 73.33 per cent disease

Table I

Effect of different treatments on disease incidence of papaya ringspot virus in papaya under field conditions.

Treatments	Percent Disease Incidence (%)	Disease control over treateed (%)
T1 = Border crop with African tall maize	26.67	73.33
T2 = Reflective mulch	34.33	65.67
T3 = Soil and foliar spray of micronutrients	41.00	59.00
T4 = Foliar spray of micronutrients	33.33	66.67
T5 = Spray with neem leaf extract @ 5ml / litre	46.67	53.33
T6 = Spray with neem oil @ 5ml / litre	40.00	60.00
T7 = Spray with*Kappaphycus alvarezii*–1 @ 4ml / litre	28.37	71.66
T8 = Spray with *Kappaphycus alvarezii*–2 @ 6ml / litre	39.23	60.77
T9 = Spray with Synthetic Oligo @ 0.25mg / litre	28.67	71.33
T10 = Combination of all the above treatments	13.33	86.67
T11 = Control	100.0	- - - -
S.E.M	0.215	- - - -
CV	23.94	- - - -
CD @ 1%	0.621	- - - -

TABLE II

Effect of different treatments on growth and yield parameters of PRSV infected papaya underfield conditions

Treatments	Average plant height (feet)*	No. of fruits per plant *	Average yield per plant (kg)*	Total Yield / ac (tons)	Yield increase over control (%)
T1 = Border crop with African tall maize	3.86	8.67	46.23	57.04	56.58
T2 = Reflective mulch	3.49	6.00	41.57	51.29	51.72
T3 = Soil and foliar spray of micronutrients	3.59	7.00	45.20	55.77	55.59
T4 = Foliar spray of micronutrients	3.37	6.67	40.87	50.43	50.89
T5 = Spray with neem leaf extract @ 5ml/litre	3.39	6.64	42.47	52.41	52.74
T6 = spray with neem oil @ 5ml/litre	3.56	6.93	41.10	50.72	51.17
T7 = Spray with *Kappaphycusalvarezii* –1@ 4ml/litre	3.48	7.43	41.87	51.66	52.06
T8 = Spray with *Kappaphycusalvarezii* –2 @ 6ml/litre	3.64	7.67	43.77	54.01	54.15
T9 = Spray with Synthetic Oligo @ 0.25mg/litre	3.34	6.67	40.90	50.47	50.92
T10 = Combination of all the above treatments	4.40	9.67	48.52	59.87	58.63
T11 = Control	3.00	3.3	20.07	24.76	----
S.E.M	0.197	0.593	0.773	----	----
CV (%)	9.460	14.37	3.256	----	----
CD @ 1%	0.5819	1.749	3.112	----	----

*Average of two locations

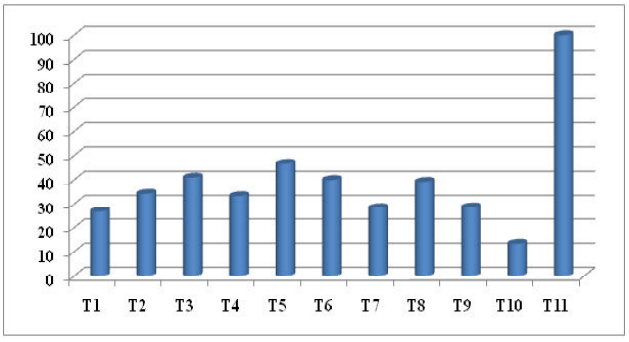

Fig 1: Effect of different treatments on disease incidence of Papaya ringspot virus in papaya under field conditions

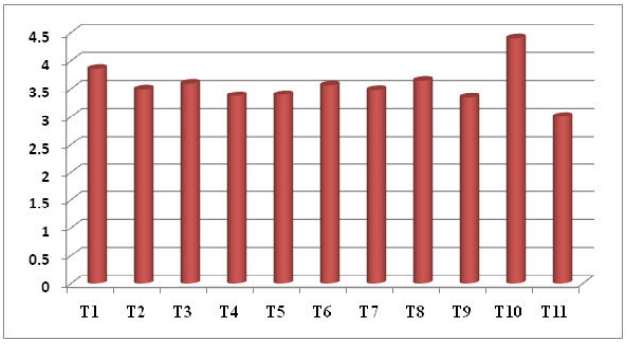

Fig 2: Effect of different treatments on plant height of Papaya ringspot virus in papaya under field conditions.

reduction (Fig 1), This is because the barrier crops have been shown to be effective in reducing virus transmission in crops by blocking aphids from reaching the target plant. Since the barrier crop can grow upto 10-11 feet, it can be suggested as intercrop with widely grown dwarf and semi dwarf varieties like, Red Lady. Diversification of flora can reduce the incidence of many non-persistent aphid-borne viruses. Because,

Plate 2: Combination of all the treatments (T10)

Plate 3: Control plants (T11)

non-host crops attenuates the spread of non persistent viruses by avoiding influx of vector population on main crop (Krishna Kumar *et al*., 2010). Hence, barrier crops like Jowar / Maize should be sown densely along the perimeter two months before papaya transplanting.

The physical approach of growing papaya with silver reflective row (T2) covers recorded 65.67 per cent disease control with an average plant height and yield of 3.49 feet and 41.57 kg per plant respectively. Reflective or floating row covers delay the appearance of virus diseased plants by excluding or repelling the aphids by reflecting UV light (Gonsalves *et al*., 2010). Compare to other colors of plastic mulch, silver reflective mulch recorded superior in reducing aphid populations.

Soil and foliar application of micronutrients (T3) was found significantly superior over foliar spraying of micronutrients which has recorded 3.59 feet of plant, 45.20 kg of yield per plant and 41.00 per cent disease control. Yadav *et al*. (2010) recorded maximum plant growth and minimum crop duration with recommended dose of fertilizers (200+90+200 NPK g / plant) + 40 g Zn EDTA + 20 g MnSO4 + 5 g CuSO4 + 10 g Borax / plant.

Among the bio-molecules, *Kappaphycusalv arezii*-1 (T7) has recorded 71.66 per cent disease control which was followed by Synthetic Oligo (SEVI) *i.e*., 71.33 per cent. The seaweed (marine macroalgae) polysaccharides such as laminarin, sulphated laminarin, fucans and lambda carrageenan and oligosaccharides such as oligocarrageenan kappa, the oligo-alginate Poly-Ma and the oligosulphated-galactan Poly-Ga also stimulate defense responses in plants inducing protection against bacteria, fungi and viruses (Laporte *et.al*., 2007). It was also shown that the seaweed oligo-sulphated-galactan Poly-Ga induced protection against tobacco mosaic virus (TMV) in tobacco plants.

Spraying with bio-pesticides like neem leaf extract and neem oil has recorded 53.33 and 60.00 per cent respectively, which were significantly superior over control which has recorded 100 per cent disease incidence (Plate 3). In recent years, plant extracts are of great concern for plant protection against different diseases (Harish *et al*., 2008; Slusarenko *et al*., 2008 and Madhusudhan *et al*., 2011). The AVP substances from plant extracts were found to be effective in reducing the Sunflower necrosis virus (SFNV) infection both in cowpea and sunflower plants (Lavanya *et al*., 2009).

Though the disease was known from quite a long period, no effective disease control measures have been developed. Hence, sustainable and non chemical integrated management method which comprises of growing maize as live barriers, physical approach of reflective mulch, application of micronutrients, use of biopesticides and biomolecules provides an efficient method against papaya ringspot virus control.

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(*Received :* May, 2017 *Accepted :* August, 2017**)**