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Efficacy of coconut water liquid formulation of *Pseudomonas aeruginosa* Migula

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Coconut water formulation of *Pseudomonas aeruginosa* Migula at 1.5 per cent concentration was found to be very effective against *Pyricularia grisea* (83.31 %), *Alternaria solani* (83.14 %), *Macrophomina phaseolina* (65.86 %) and *Sclerotium rolfsii* (64.41%) showing maximum growth inhibition. Significant reduction in Per cent Disease Intensity of blast finger millet of recorded when the crop was sprayed with 1 per cent coconut water formulation (6.79 PDI) three times. Significant higher germination (96.25 %), lower seed rot (3.75 %) and stem rot incidence (15.61 %) were recorded in groundnut, when application of 10 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g of vermicompost was applied in sick soil.

Key words: *Pseudomonas aeruginosa,* Coconut water, finger millet, groundnut

INTRODUCTION

Development of liquid formulation which ensures quality product in liquid over conventional carrier based biofertilizers and biopesticides are very important. These liquid formulations facilitate long shelf life up to 2 years, convenience of handling, storage and transportation, easy quality control, more export potentials and also being preferred by the community of farmers as well as manufacturers. In liquid formulation, the microbial organism are present in a dormant cyst form and after application in the field, the dormant form gives to active cell. This help to increase the shelf life of liquid formulation for more than 1 year (Vendan and Thangaraju, 2007). Recently, Kolombet *et al.*, (2008) have reported the extended shelf life of a *Trichoderma asperellum* in liquid formulation. Coconut liquid endosperm, though nutritive in tender stages, become depleted of nutrient as the nut matures. From mature nut the liquid endosperm is seldom used. Mature coconut when split open for making copra, the coconut water is discarded as an agricultural waste which can be used as a good medium for mass multiplication of bioagents (Jayelekshmy *et al.*, 1988, Nathanael, 1996). Hence, in present study cheap natural media i.e. coconut water based liquid formulation of *P. aeruginosa* has been used for *in vitro* and *in vivo* test.

MATERIALS AND METHODS

Preparation of coconut water based liquid formulation of P. aeruginosa

Sterilized conical flasks of 250 ml were taken and 100 ml of distilled sterilized water was filled. Coconut water was added @ 30 ml in the flasks. Further, the flask was sterilized in autoclave at 121^0C for 15 min. After cooling, 0.1 ml of *P. aeruginosa* suspension (2×10^9 cfu/ml) was transferred to each flask and they were incubated at 28 ± 2^0C temperature.

Testing the efficacy of coconut water formulation against different pathogens in vitro

The *in vitro* antagonistic bio-efficacy of liquid formulation using mature coconut water was determined by poison food technique (Nene and Thapliyal, 1979). Coconut water formulation of *P. aeruginosa* (48 h) was used @ 2, 5, 7, 10 and 15 ml were added in 1 liter of PDA medium after melting and cooling. Twenty ml PDA were poured in 90 mm Petri dish. After solidifying the media, the mycelial bits were taken from 5 days old culture of the pathogens by 5 mm sterilized cork borer. The bits were placed at the centre of plate and incubated at room temperature (28±2^{0}C).

The growth inhibition were recorded after 5th days of inoculation.

Per cent growth inhibition was calculated using the following formula suggested by Vincent, (1927),

$$PGI = \frac{C-T}{C}$$

where,

PGI = Per cent growth inhibition
C = Average diameter of mycelial growth in control (mm).

T = Average diameter of mycelial growth in treated set (mm).

Testing the efficacy of coconut water formulation of P. aeruginosa : Blast of finger millet

The variety, GN-4 is the most commonly cultivated variety of south Gujarat, which is highly susceptible to blast, was selected for the experiment and experiment conducted in green house with five treatments and four repetitions. 45 x 75 cm sterilized pots were filled with sterilized soil. The seedlings of 21 days old of GN-4 variety were transplanted in the pots. The suspension of the pathogen (10^6 conidia/ml) was sprayed on seedling 21 days after transplanting to create the blast infection. Coconut water formulation of *P. aeruginosa* was sprayed after 10 days of pathogen inoculation @ 2, 5, 7 and 10 ml/ lit. of water. Control in which only water was sprayed. Observations by using 0-5 scale were recorded after seven days of each spray (Nagraja *et al.*, 2007). Three sprays were given at 15 days interval and Per cent Disease Intensity was calculated by using following formula,

$$PDI = \frac{\Sigma \text{ of ratings of infected leaves observed} \times 100}{\text{No. of leaves observed} \times \text{Maximum disease score}}$$

Stem rot of groundnut

The variety GG 2 is the susceptible variety to this disease and hence was selected to test the formulation against this soil borne problem. Experiment conducted in green house with five treatments and four repetitions. Earthen pots of 45 x 75 cm were filled with sterilized soil. The pathogen inoculum (which is multiplied in sand maiz meal medium) was applied @ 50 g/kg of soil. Such sick soil then filled in pot @ 5 kg/pot. The five seeds of groundnut were sown in each pot after a day of inoculation. Coconut water liquid formulation of *P. aeruginosa* (2, 5, 7 and 10 ml) was mixed in 50 g of vermicompost and was applied in a pot after 3 days of soil inoculation with *S. rolfsii.* Seed emergence and seed rot was calculated 10 days after sowing. Stem rot incidence was recorded regularly up to 45 days after sowing (DAS) by counting the infected plants. Per cent Disease Incidence was calculated by using following formula,

$$\text{Disease Incidence(\%)} = \frac{\text{No. of infected plants} \times 100}{\text{Total No. of plants}}$$

RESULTS AND DISSCUSION

Efficacy of coconut water formulation against different pathogens *in vitro*

Pyricularia grisea

Minimum mycelial growth of the pathogen (10.05 mm) was recorded in the treatment of 1.5 per cent of coconut water formulation which was at par with 1 (10.12 mm) and 0.7 per cent coconut water formulation (10.22 mm). Next treatment was 0.5 (10.30 mm) followed by 0.2 per cent coconut water formulation (10.40 mm). Maximum growth inhibition was recorded in 1.5 per cent coconut water formulation (83.31 %), followed by 1 (83.19 %), 0.7 (83.02 %), 0.5 (82.90 %) and 0.2 per cent coconut water formulation (82.73 %). (Table 1)

Alternaria solani

Minimum mycelial growth of the pathogen (10.22 mm) was recorded in treatment of 1.5 per cent in

Table 1 : *In vitro* efficacy of coconut water based formulation of *P. aeruginosa* against various pathogens after 5thday

Concentration (%)	*Pyricularia grisea*		*Alternaria solani*		*Macrophomina phaseolina*		*Sclerotium rolfsii*	
	Average colony diameter (mm)	Per cent inhibition over control	Average colony diameter (mm)	Per cent inhibition over control	Average colony diameter (mm)	Per cent inhibition over control	Average colony diameter (mm)	Per cent inhibition over control
0.2	10.40	82.73	10.65	82.44	70.35	21.72	53.70	37.44
0.5	10.30	82.90	10.57	82.56	40.67	54.74	42.37	50.64
0.7	10.22	83.02	10.42	82.81	35.52	60.42	33.17	61.35
1.0	10.12	83.19	10.30	83.01	33.25	63.00	32.67	61.93
1.5	10.05	83.31	10.22	83.14	31.57	64.86	30.55	64.41
Control	60.25		60.65		89.87		85.85	
S.Em.±	0.11		0.03		0.05		0.16	
C.D. at 5%	0.32		0.10		0.16		0.49	
C.V.%	1.19		0.38		0.22		0.72	

coconut water formulation after 5 days of incubation which was at par treatment of 1 per cent coconut water formulation (10.30 mm). Next treatment was 0.7 (10.42 mm) followed by 0.5 (10.57 mm) and 0.2 per cent coconut water formulation (10.65 mm). Maximum growth inhibition was recorded in 1.5 per cent coconut water formulation (83.14%) followed by 1 (83.01 %), 0.7 (82.81 %), 0.5 (82.56 %) and 0.2 per cent coconut water formulation (82.44 %). (Table 1)

Macrophomina phaseolina

Minimum mycelial growth of the pathogen (31.57 mm) was recorded in the treatment of 1.5 per cent of coconut water formulation which was significantly better over all the rest treatments. The next was 1 per cent coconut water formulation (33.25 mm) followed by 0.7 (35.52 mm), 0.5 (40.67 mm) and 0.2 per cent coconut water formulation (70.35 mm). These were comparatively less effective and resulted higher in growth of pathogen. Maximum growth inhibition was recorded in 1.5 per cent coconut water formulation (64.86 %) followed by 1 (63.00 %), 0.7 (60.42 %), 0.5 (54.74 %) and 0.2 per cent coconut water formulation (21.72 %). (Table 1)

Table 2 : Effect of coconut water formulation of *P. aeruginosa* on finger millet leaf blast in pot experiment

Concentration (%)	Percent Disease Intensity after seven days of spray		
	First spray	Second spray	Third spray
0.2	14.77 (6.51)	18.62(10.22)	20.07(11.79)
0.5	12.98(5.06)	18.42(10.00)	19.20(10.84)
0.7	12.25(4.55)	16.71(8.30)	16.65(8.22)
1.0	10.20(3.15)	16.10(7.71)	15.10(6.79)
Control	16.51(8.09)	21.79(13.80)	22.52(14.69)
S.Em.±	0.33	0.37	0.18
C.D. at 5%	1.01	1.11	0.55
C.V.%	5.03	4.05	1.96

*Figure outside the parenthesis are original values, those inside the parenthesis are Arc sine transformation values.

Table 3 : Effect of coconut water formulation of *P. aeruginosa* on stem rot of groundnut in pot experiment

Concentration (%)	Seed emergence (%)	Seed rot (%)	Stem rot (%)
2ml/50 g of vermicompost	61.82 (77.50)	28.12 (22.50)	49.61 (58.05)
5ml/50 g of vermicompost	67.32(85.00)	22.63 (15.00)	40.74 (42.63)
7ml/50 g of vermicompost	72.91 (91.25)	18.13 (10.00)	28.13 (22.25)
10ml/50 g of vermicompost	81.48 (96.25)	8.47 (3.75)	23.25 (15.61)
Control	47.14 (53.75)	36.03 (35.00)	59.34 (74.02)
S.Em.±	2.92	2.89	0.51
C.D. at 5%	8.81	8.71	1.55
C.V.%	8.25	25.49	2.57

Sclerotium rolfsii

Minimum mycelial growth of the pathogen (30.55 mm) was recorded in the treatment of 1.5 per cent of coconut water formulation which was significantly superior over all the treatment. The next treatment was 1 per cent coconut water formulation (32.67 mm) followed by 0.7 (33.17 mm), 0.5 (42.37 mm)

and 0.2 per cent coconut water formulation (53.7 mm). Maximum growth inhibition was recorded in 1.5 per cent coconut water formulation (64.41 %), followed by 1 (61.93 %), 0.7 (61.35 %), 0.5 (50.64 %) and 0.2 per cent coconut water formulation (37.44 %). (Table 1)

Hence, 1.5 percent concentration was found very effective against *P. grisea, A. alterana, M. phaseolina* and *S. rolfsii* in *in vitro*. Sharma *et al.* (2007) found significant antifungal activity of the *P. aeruginosa* (multiplied in nutrient broth) against *Fusarium moniliformae, A. solani* and *Helminthosporium holodes* and found that the strain was effective in inhibiting mycelial growth.

In vivo

Blast of finger millet

There was significant reduction in the disease intensity of leaf blast of finger millet in all the concentrations of the coconut water formulation tested after 7 days of each spraying as compared to the control. Among this, significantly lower leaf blast intensity was recorded in 1 per cent coconut water formulation of *P. aeruginosa* (6.79 PDI) followed by treatment 0.7 (8.22 PDI), 0.5 (10.84 PDI) and 0.2 per cent coconut water formulation of *P. aeruginosa* (11.79 PDI) as compared to the control (14.69 PDI). The results were more or less similar with the Sitter and Gnanamanickam (1996) who emphasized six strains of *P. fluorescens* and *P. putida* showed fungal inhibition in dual assay *in vitro* and reduced *Elusine coracana* blast severity in the field. It is reported that seed treatment with *P. fluorescens* Pf2 (0.6%) and two foliar sprays at 10 days interval reduced blast intensity in *E. coracana*. (Table 2).

Stem rot of groundnut

Seed emergence

There was significantly higher germination of groundnut seeds in all the treatment as compared to the control. Among this, significantly higher seed emergence was recorded in the application of 10 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (96.25 %). Next best treatment in order of merit was 7 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (91.25 %), followed by 5 ml (85.00 %) and 2 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (77.50 %) (Table 3).

Seed rot

Significantly lower seed rot incidence was recorded in the application of 10 ml of coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (3.75 %). Next best treatment in order of merit was 7 ml (10.00 %), followed by 5 ml (15.00 %) and 2 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (22.50 %). It was the lowest in untreated control (35.00 %)(Table 3).

Stem rot

Significantly lower stem rot incidence was recorded in the application of 10 ml of coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (15.61 %). Next best treatment in order of merit was 7 ml (22.25 %), followed by 5 ml (42.63 %) and 2 ml coconut water formulation of *P. aeruginosa* multiplied in 50 g vermicompost (58.05 %) as compared to the control (74.02 %) (Table 3).

The reduction of disease by *Pseudomonas* may be due to (i) application of *Pseudomonas* isolates strengthen host cell wall structures resulting in restriction of pathogen invasion in plant tissue (ii) activation of enzymes of phenylpropanoid metabolism and accumulation of PR-proteins in finger millet leaves (Radjacmmare *et al.*, 2004a) (iiii) active induce systemic resistance (iv) induction of defense protein viz. chitinase, β-1,3 glucanase, peroxidase (PO) and polyphenol oxidase (PPO) (v) increase amount of silicic acid in the leaves and (vi) expression of defense gene against finger millet blast, (Radjacmmare *et al.*, 2004b).

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