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Botanical Formulation of *Muntingia calabura* for the Management of Early Leaf Blight in Tomato

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The present study was successful in demonstrating the inhibitory activity of the medicinal plant *Muntingia calabura* against *Alternaria Solani* that causes early blight of tomato and proposes the development of a new botanical formulation (Muntingin 5EC) and its use in plant disease management after package and practice. This ecofriendly botanical formulation was developed from the purified antimicrobial metabolite (Stigmasterol) isolated from the methanol extract of *M. calabura* root. Different concentrations of Muntingin 5EC was examined on seed infection, germination and seedling vigour of tomato and it was found that two per cent Muntingin 5EC increased the germination and vigour and reduced the early leaf blight infection in tomato seed to a significant extent. Application of Muntingin 5EC increased the enzymes activity such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and phenol content of tomato. Muntingin 5EC (2%) was found to be the optimum concentration for the control of early blight of tomato under pot culture conditions. Application of this botanical in plant disease management assumes special significance by being an ecofriendly and cost effective strategy, which can be used in integration with other strategies for a greater levels of protection with sustained crop yields after sufficient evaluation.

Key words: *Alternaria solani,* Botanical fungicide, *Muntingia calabura,* Muntingin 5EC*,* Plant diseases management*.*

Plant diseases cause considerable losses in crop production and storage. The intensive and indiscriminate use of pesticides and fungicides in agriculture causes many problems such as polluting the environments like water and soil, and residual contamination of food and many others in addition to socio- economic problems (Stangarlin *et al*., 1999). Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides and fungicides. In this context, biological control through the use of natural antagonistic microorganisms has emerged as a promising alternative (Strange and Scott, 2005). Botanicals with antifungal compounds have been identified and these can be exploited for the management of diseases (Kagale *et al*., 2004). Botanicals have low mammalian toxicity, target specificity, biodegradability and contain many active ingredients in low concentrations, thus possess biocidal activity against several insect pests and pathogens (Kalaycioglu *et al*., 1997; Harish *et al*., 2008).

Crude extracts of some well known medicinal plants are used to control the plant pathogens. During the past few years, there is a growing trend all over the world to shift from synthetic to natural products including medicinal plants (Parimala devi and Marimuthu, 2011). The neglected and little known botanicals should be considered now to cure the plant diseases, which create challenging problems in agriculture and pose real economic and environmental threats. So it is better to opt for a natural productivity to cure the plant diseases which is usually economical and minimize environmental threats.

Early blight disease of tomato by *Alternaria solani* has become most destructive in India and yield losses due to this pathogen was up to 80% (Singh, 1985; Mathur and Shekhawat, 1986; Chandravanshi *et al*., 1994; Shanmugasundaram, 2004). The control of tomato early blight disease has been exclusively based on the application of chemical pesticides. Several effective pesticides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. In an attempt to modify this condition, some alternative methods have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases. Use of plant products and biocontrol agents has been shown to be eco-friendly and effective against many plant pathogens. A number of plant species have been reported to possess natural substances that are toxic to many fungi causing plant diseases (Lee *et al*., 2007). *Muntingia calabura* L. (Kerukup siam), also known locally as

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Jamaica cherry, is a plant of the family Elaeocarpaceae. The leaves, barks and flowers are believed to possess medicinal value as reported in Peru folklore medicinal uses. The objective of the present study is to evaluate the antimicrobial activity of root extracts from *Muntingia calabura* against *A. solani* under *in vitro* conditions and to develop a botanical formulation against plant diseases control.

Materials and Methods

Preparation of the botanical fungicide

The partially purified methanol extract of *M. calabura* root containing the antimicrobial metabolite Stigmasterol was used to develop emulsifiable concentrates. The condensed material containing the antimicrobial metabolite, obtained after column chromatographic separation of fractions was considered as 100 per cent concentration. The formulation was developed by using recommended quantities of surfactant (Tween20) and co-surfactant (Ethylmethyl ketone). The 4EC formulation were prepared by adding 4 g of antimicrobial metabolite to 20 ml methanol and made up to 100 ml by adding 10 ml of tween20 and 70 ml of Ethylmethyl ketone. The active fraction from methanol extract of *M. calabura* root was developed into emulsifiable concentrate and designated as 'Muntingin 5EC'. Muntingin 5EC at different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5%) were tested for the efficacy under *in vitro* conditions.

Assessing the biocontrol potential of Muntingin 5EC against tomato early leaf blight

Effect of Muntingin 5 EC on seed infection, seed germination and vigour of tomato seedlings were evaluated under *in vitro* condition. The treatments adopted were Muntingin 5EC at five different concentration (0.2 %; 0.4%; 0.6%; 0.8%; and 1.0%), 0.2% Mancozeb (pesticide control) and standard biocontrol agent (*P. fluorescens* PF1). The tomato seeds were soaked in different concentrations of Muntingin5EC for 2 h and twenty five seeds of each treatment were placed on moist blotters (ISTA, 1993) in petriplate and incubated at 20 ± 2°C for 12 h of alternate natural light and 12 h of darkness. The seeds were examined for growth of seed borne pathogens on eighth day of treatment. The seed infection was expressed in percentage. The seedlings were evaluated as total number of normal seedlings and evaluating the per cent germination. The Vigour Index was compared and expressed as whole number.

Evaluation of Muntingin 5EC on tomato early leaf blight under pot culture condition

Effect of different concentrations of Muntingin 5EC (0.5, 1.0, 1.5 and 2.0%) along with standard practices of Mancozeb (0.2%) and biocontrol agent (*P. fluorescens* PF1) on early blight (Caused by *A. solani*) was tested under pot culture condition in tomato. The extent of disease incidence is expressed as per cent disease index (PDI) and calculated using the disease score card for *A. solani* (Ayyangar, 1928). The peroxidase (Puttur,1974), polyphenol oxidase (Mayer *et al.*, 1965) and Phenylalanine ammonia lyase (Zucker, 1965) were analysed in the roots of tomato upto 10 days at 2 days interval. The phenol content was also measured at two days interval by standard procedure as described by (Spies, 1955).

Results and Discussion

The biocontrol formulation prepared using partially purified methanol extract of *Muntingia calabura* containing antimicrobial metabolite stigmasterol was assessed in this study for its biocontrol potential. The prepared formulation tested for its efficacy in controlling the early leaf blight disease of tomato under *in vitro* and pot culture studies.

Effect of Muntingin 5EC on seed infection, seed germination of tomato seedlings

The seed infection by *A. solani* was reduced by 99.28 per cent in Muntingin 5EC (2%) treated seeds. The treatments which received Mancozeb (0.2 %) and *P. fluorescens* recorded 89.28 and 90.21 per cent reduced seed infection respectively over the control. The germination per cent was increased by 16.50 per cent in Muntingin 5EC (2%) treated seeds compared to the control (Table 1).

The effect of various concentrations of Muntingin 5EC on growth of tomato seedlings is presented in Table 2. The maximum shoot (13.06 cm) and root length (10.25 cm) were observed in the seeds which received 2.0 per cent Muntingin 5EC. The 2.0 per cent Muntingin 5EC treated plants recorded 71.84

Table 1. Effect of Muntingin 5EC on disease infection of seed and seed germination of tomato (PKM1)

Treatment	Disease infection of Seed		Seed germination	
	Infection (%)*	Reduction over control	Germination (%)*	Increase over control
T_1 - 0.50% Muntingin 5EC	10.20 (± 0.05)$_{b}$	88.79	83.00	3.75
T_2 - 1.00% Muntingin 5EC	7.30 (± 0.01)$_{cd}$	91.97	85.80	7.25
T_3 - 1.50% Muntingin 5EC	4.00 (± 0.06)$_{cd}$	95.60	89.65	12.06
T_4 - 2.00% Muntingin 5EC	0.65 (± 0.02)$_{d}$	99.28	93.20	16.50
T_5 - Mancozeb 0.2%	9.75 (± 0.01)$_{bc}$	89.28	83.50	4.37
T_6 - *P. fluorescens*	8.90 (± 0.09)$_{cd}$	90.21	84.10	5.12
T_7 - Control	91.00 (± 0.02)$_{a}$		80.00	

Values are mean (± SE) of five replicates and values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test ($p < 0.05$)

and 43.35 per cent increased shoot and root length respectively over the control. The vigour index was also maximum with 2.0 % Muntingin 5EC treatment. The treatments which received Mancozeb and *P. fluorescens* recorded 7.70 and 11.59 per cent increased vigour index respectively over control.

Table 2. Effect of Muntingin 5EC on vigour of tomato seedlings

Treatment	Shoot length (cm)*	Per cent increase over control	Root length (cm)*	Per cent Increase over control	Vigour index *	Per cent Increase over control
T_1 - 0.50% Muntingin 5EC	10.08 (± 0.02)$_b$	32.63	9.00 (± 0.01)$_{ab}$	25.87	1583.64	39.45
T_2 - 1.00% Muntingin 5EC	11.50 (± 0.12)$_{ab}$	51.31	10.40 (± 0.12)$_a$	45.45	1879.02	55.78
T_3 - 1.50% Muntingin 5EC	12.75 (± 0.01)$_a$	67.76	10.55 (± 0.02)$_a$	47.55	2088.84	70.08
T_4- 2.00% Muntingin 5EC	13.06 (± 0.02)$_a$	71.84	10.25 (± 0.01)$_a$	43.35	2172.49	76.29
T_5 - Mancozeb 0.2%	8.18 (± 0.06)$_c$	7.63	8.00 (± 0.02)$_b$	1.18	1351.03	7.70
T_6 - *P. fluorescens*	8.30 (± 0.23)$_c$	9.21	8.25 (± 0.06)$_b$	1.53	1391.85	11.59
T_7 - Control	7.60 (± 0.01)$_c$		7.15 (± 0.09)$_b$		1180	

Values are mean (± SE) of five replicates and values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test (p< 0.05)

Effect of Muntingin 5EC on early blight control in tomato

The tomato plants sprayed with various concentrations of Muntingin 5EC under pot culture studies were observed for the early blight disease incidence. The per cent disease index of the Muntingin 5EC treated plants was significantly lesser when compared to the control. The treatment which received 2 per cent Muntingin 5EC recorded 99.4 per cent reduced PDI when compared to control. This was followed by the treatment 1.5% Muntingin 5EC which recorded 98.07 per cent reduced PDI, whereas the treatment which received mancozeb (0.2%) and *P. fluorescens* recorded 94.89 and 93.81 per cent reduced PDI respectively (Table 3).

Effect of Muntingin 5EC spray on plant defence enzymes in roots of tomato

The changes in activities of various enzymes were monitored on 0, 2, 4, 6 and 10 days after the inoculation of *A. solani*. The PO activity reached maximum at 6 days after inoculation with 2.0 per cent Muntingin 5EC treated plants (2.40 units/g of leaf tissue) and then declined thereafter. Though the peroxidase activities increased in inoculated control, the values were significantly lower than the plants treated with various concentrations of Muntingin 5EC, Mancozeb (0.2%) and *P. fluorescens*. In inoculated control, the activity reached maximum (1.80 units/g of leaf tissue) on second day of inoculation and then declined (Fig. 1a).

Table 3. Effect of Muntingin 5EC on per cent disease index of early blight of tomato under pot culture condition

Treatment	Percent disease index*	Percent reduction over control
T_1 - 0.50% Muntingin 5EC	10.83 (± 0.01)$_b$	83.96
T_2- 1.00% Muntingin 5EC	6.56 (± 0.02)$_c$	90.28
T_3 - 1.50% Muntingin 5EC	3.45 (± 0.26)$_d$	94.89
T_4 - 2.00% Muntingin 5EC	0.36 (± 0.02)$_e$	99.46
T_5 - Mancozeb 0.2%	1.30 (± 0.03)$_e$	98.07
T_6- *P. fluorescens*	4.18 (± 0.01)$_d$	93.81
T_7 - Uninoculated control	-	
T_8 - Control	67.55 (± 0.03)$_a$	

Values are mean (± SE) of five replicates and values followed by the same letter in each column are not significantly different from each other as determined by Duncan's multiple range test (p< 0.05)

Similarly, the polyphenol oxidase activity increased in plants treated with Muntingin 5EC when compared to inoculated control. Muntingin 5EC at 2.0 per cent concentration significantly increased the activity of PPO to maximum level (2.72 units/g of leaf tissue) on tenth day of inoculation (Fig. 1b). Among the treatments, maximum phenylalanine ammonia lyase activity was observed (49.00 units/ g of leaf tissue) with 2.0 % Muntingin 5EC (Fig. 1c). The concentration of phenol was significantly higher in plants treated with 2.0 per cent Muntingin 5EC (188.4 ìg of catechol/g of leaf tissue) than all other treatments on sixth day of challenge inoculation. In all the treatments, maximum phenol content reached on sixth day after inoculation. However, all the treatments retained the content of phenol without much reduction even on tenth day, but the phenol content reduced drastically in inoculated control (Fig. 1d).

Botanicals are materials or products of plants origin valued for their pesticidal, medicinal or therapeutic properties. Phytopesticide materials range from whole fresh plants to purely isolated bioactive phytochemicals or their formulations which are effective against pests and pathogens (Prakash and Rao, 1996). Preparation and application of botanicals for crop protection are linked to the folklores and tradition of the farmers (Anjorin, 2008). Management of disease through fungicides alone leads to cause soil residual problem and health hazards, besides involving higher input cost. One of

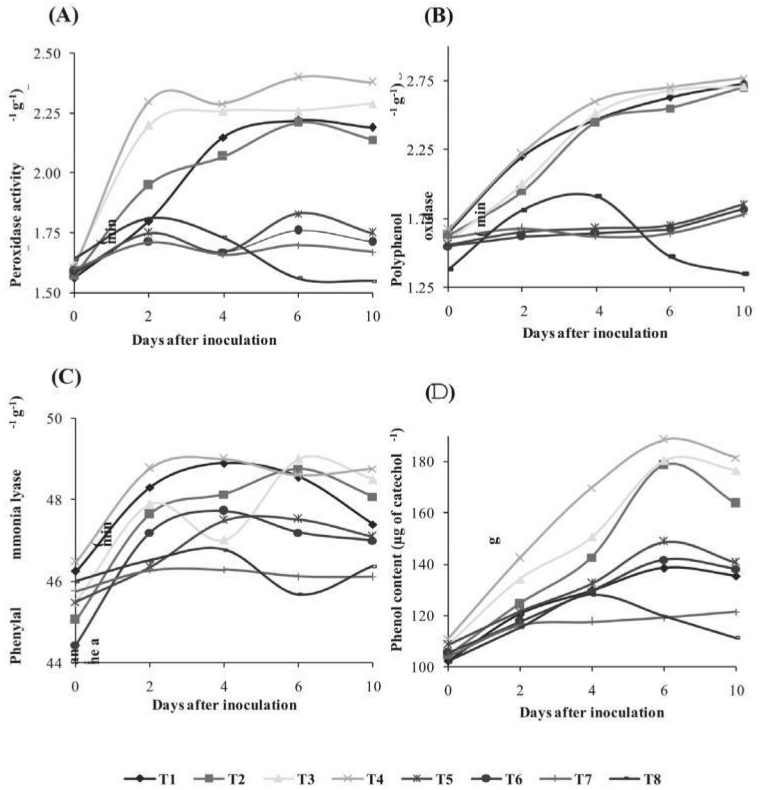

Fig. 1. Effect of Muntingin 5EC on Changes in plant defense metabolites of tomato under pot culture condition. A-Peroxidase; B- Polyphenol oxidase; C- Phenylalanine ammonia lyase; D- Phenol content ; T_1 -T_8 : as described in Table 3.

the recent approaches for plant disease management is exploitation of plant products. Inspite of the wide recognition that many plants possess antimicrobial properties, only a handful of products have been developed, because of their less persistence nature in the crop ecosystem (Kumbhar *et al*., 2001).

As part of the present study, Emulsifiable Concentrate (EC) formulation was developed from the partially purified antimicrobial compound obtained from methanol root extract of *M. calabura*. The formulation was prepared as 4EC and 5EC with a naming as 'Muntingin'. The botanical formulation was prepared with 5EC concentration with account of 2 per cent formulation should contain 1 mg of the active compound per ml of the product which is the actual MIC value for the antimicrobial compound stigmasterol.

Parimala devi and Marimuthu (2011) reported that the botanical formulation, Polymin 40 EC (P-40) at various concentrations (0.5, 1.0, 1.5 and 2.0 per cent) controlled *A. solani* . The P-40 at 2.0 per cent effectively controlled the pathogens under pot culture conditions and was considered as the optimum concentration. The botanical formulation at 2 per cent level was found to reduce the seed infection of *Fusarium oxysporum* f.sp. *lycopersici* in tomato and also increased the vigour of tomato (76.29%) when compare to the control.

The present investigation, the botanical formulation**,** Muntingin 5 EC at various concentrations (0.50, 1.00, 1.50, and 2.00 per cent) was tested under pot culture conditions in controlling *A. solani* in tomato. The Muntingin 5 EC at 2.0 per cent level effectively controlled the pathogen under pot culture conditions and was considered as the optimum concentration. The increase in germination percentage and vigour of seedlings may be due to the reason that the application of Muntingin promoted the activity of seed enzymes such as amylase, catalase, etc. and also increased the metabolic activities of the seed.

The biotic and abiotic inducers play an important role in activating the defense genes in plants. Induction of defense proteins makes the plant resistant to pathogen invasion (Ramanathan *et al*., 2000). The results of the present study revealed that the tomato applied with Muntingin 5 EC significantly induced the defense compounds (PO, PPO, PAL and Phenol) compared to control. The resistance of plants induced against the pathogens, due to the application of botanicals has been widely reported (Straub *et al.,* 1986; Rajeswari, 2002). Kagale *et al*. (2004) observed an increase in antioxidant enzymes in rice plants sprayed with *Datura metal* leaf extract. Increase in PO activity has been observed in the sesame plants treated with *Azadirachta indica* leaf extract (Guleria and Kumar, 2006). Plant phenolics are well known antifungal, antibacterial and antiviral compounds that increase the physical and mechanical strength of the host cell wall. Phenylalanine ammonia lyase is the first enzyme of phenyl propanoid metabolism in higher plants and has been suggested to play a significant role in regulating the accumulation of phenolics (Daayf *et al*., 1997). The formulation Muntingin 5EC containing the antimicrobial compounds led to increased biosynthesis of PO, PPO, PAL and phenols, which in turn were responsible for disease resistance in plants.

It has been concluded that the botanical formulation "Muntingin" increased the tomato germination percentage by reducing the seed infection by *A. solani*. It has also been showed the potential inhibitory effect on the selected plant pathogen under pot culture conditions. The botanical formulation is capable of inducing the resistance in tomato plants through enhancement of defense compounds. Thus the formulation Muntingin showed the potential for managing the early leaf blight disease in tomato. The future thrust and follow-up research efforts may aim to study the effect of Muntingin on plant disease control under field conditions and this will provide an opportunity for eco-friendly disease management on a variety of crops.

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