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R Parimala Devi
National Pulses Research Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

P Marimuthu
Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
R Parimala Devi
National Pulses Research Centre, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Stability studies on newly developed botanical formulation: Polymin 40 EC

R Parimala Devi and P Marimuthu

Abstract
Use of botanicals in plant disease management is getting popular nowadays due its merits over the environment and agricultural products. An ecofriendly botanical formulation "Polymin 40 EC" was developed from the chloroform extract of *Polygonum minus* Huds which possessed broad spectrum antimicrobial activity against plant pathogens. The stability in addition to its activity is more important for a plant protection chemical and hence the stability of the newly developed botanical formulation "Polymin 40 EC"was studied in detail. The emulsion stability and thermostability analysis revealed that "Polymin 40 EC" was found to be more stable and 2% "Polymin 40 EC" was found to have a neutral pH. The formulations were also found to retain their antimicrobial activity upto a period of three months.

Keywords: Polymin, botanical formulation, stability

Introduction
In the present scenario of agriculture, a shift from inorganic to organic is gaining importance due to the increasing concern over health and environment among humans. The scientists and policy makers in hands with farmers are targeting for sustainable agriculture to feed the growing world. On the other hand, pests and diseases of crop plants are posing a great threat to the agriculturists due to their increasing resistance and resurgence against most of the plant protection chemicals. Botanicals are used for plant protection measures from time immemorial without knowing the scientific fact within it. And scientists are now searching for new lead molecules responsible for the antimicrobial activity present in the botanicals. Botanicals with antimicrobial activity against a particular plant pathogen can be effectively used to control it under field condition without harming the environment and human health. India is known for its richness in plant biodiversity and most of the plants remain unexplored for its antimicrobial activity. With this view, this study performed to develop a new botanical formulation from an effective medicinal plant *Polygonum minus* Huds. Which possessed broad spectrum antimicrobial activity against plant pathogens.

Materials and Methods
Stability test of EC formulations (James Pitchai and Subramanian, 2000) [2].

Emulsion stability
About 20 to 25 ml of emulsion concentrate of *P. minus* 40 EC was added to 70 ml of standard hard water and made up to 100 ml in a beaker. The contents of the beaker were stirred with glass rod (4 revolutions per second) during the addition. The contents of the beaker were transferred immediately to a clean and dry measuring cylinder. The measuring cylinder with its contents was kept in a thermostat at room temperature for one hour. After 1 h, the volume of creamed matter at the top or the sediment at the bottom was measured.

Thermo stability test
The formulation was poured into a glass bottle until three fourth was filled. The bottle was closed airtight with colloid oil sealing wax to avoid any loss of volatile solvents and kept it in the thermostat (50°C) for 7 days. After 7 days, the volume of the creamed matter at the top or the sediment at the bottom was measured.

Cold stability test
The formulation was poured in to a glass bottle until three fourth was filled. The bottle was closed airtight with colloid oil sealing wax to avoid any loss of volatile solvents and kept it in the refrigerator (10°C) for 7 days. After 7 days, the formulation was tested for emulsion stability.

Determination of pH
The formulation (0.5 ml) was diluted in 50 ml of distilled water (1%) and pH was determined with a pH meter.

Effect of storage of Polymin 40EC for different periods at room temperature on mycelial growth of fungal pathogens
The formulation was stored at room temperature (28 ± 2°C). At 15 days interval, samples were drawn and bioassays on the inhibition of mycelial growth of the selected pathogens were carried out as described earlier up to 120 days.

Effect of Polymin 40EC on mycelial growth of the pathogens
The EC formulation at various concentrations (0.50, 1.0, 1.5, 2.0 and 2.5%) was tested for antifungal activity against the selected pathogens by poisoned food technique.

Results and Discussion
The plants have a peculiarity that besides their medicinal value, they have other utility in controlling menaces of plants themselves which thwart the attempts of man to optimize their use in controlling the pests and diseases of plants (Saxena, 1993) [9]. Plant diseases are the major biotic constraints to crop growth and cause a variety of damage and yield loss. Effective integration of approaches is required in disease management to reduce the crop loss effectively. Prior to discovery of organochlorine and organophosphate compounds, botanicals were used as important products in pest and disease management in industrialized nations (Oros and Ujvary, 1999) [6]. The resultant effect on environment due to extensive use of chemical compounds in crop protection, have led to interest in developing botanical formulations in recent times.
Recently, new approaches involving botanical fungicides are considered as an alternative to synthetic fungicides as they maintain low mammalian toxicity, target specificity and biodegradability (Kumbhar *et al*., 2001) [3]. 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.
The possibility for the development of dust and EC formulations for controlling plant diseases was known from the available informations (Narasimhan *et al*., 1998; Rajappan *et al*., 1999) [5]. According to Nagarajan (1996) [4] the emulsifiable concentrates were the most popular formulations in India and the benefits were greater than other formulations. Reports suggest that emulsifiable concentrates are the most desirable for botanical formulations. The emulsifiable concentrates are found to have the following advantages like, non-abrasive, does not plug nozzles or screens, little visible residues on treated surfaces, relatively easy to transport, handle, store and little agitation required (will not settle or separate when equipment is running).
As part of the present study, Emulsifiable Concentrate (EC) was developed from chloroform leaf extract of *P. minus*. The formulation was prepared at different EC *i.e*., 30 and 40 EC and named as 'Polymin'.
Emulsion stability of the formulations was tested with standard hard water and the results are presented in Table 20. The standard critical limits of sedimentation of emulsion formulations were taken as 2 ml. For both the formulations of *P. minus i.e*., Polymin 30EC and Polymin 40EC the sedimentation levels were not exceeding the critical limits of 2 ml (Table 1).
The 40EC formulation of *P. minus* was kept at 10° C and 50° C for 7 days and the sedimentation of emulsion was recorded. The visual observations confirmed the temperature stability of the EC formulations, as there was no sedimentation observed in high and low temperatures. The 2.0 per cent solution of EC formulation had a neutral pH (7.0). Based on the results it has been concluded that the emulsions were suitable for EC formulations. Similar type of emulsion stability was reported for the oil formulations prepared from neem (Meenakshisundaram, 1991). The optimum pH of the prepared 2 per cent formulation was found to be 7.0 and neutral pH formulations are not phytotoxic to plants.
The formulation Polymin 40EC at 2.0 per cent was tested for its stability at room temperature (28 ± 2°C) for different periods (Table 2). The results showed that the EC formulation retained its 100 per cent antifungal activity up to 90 days against the four pathogens tested *viz., A. solani. A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici.* Bharathi (2004) [1] have reported that Lowfolin 40 EC formulation was effective up to 90 days after preparation. Chandrasekaran (1996) also showed that LA-WP formulation was effective up to 90 days after preparation. Storable nature of the formulation up to 3 months at room temperature has got much practical utility as its effect was retained during the storage period (Veerasamy, 1997) [12]. According to Singh *et al*. (2001) [10] the extracts of *Adenocalymma alliaceum* after a storage period of 35 days at 10° C, retained their activity against *A. tenuissima*.
Optimum concentration of the EC formulations was standardized and their antifungal activity was also tested. Two per cent Polymin 40 EC formulation was found to be more effective in controlling the fungal pathogens *viz., A. solani, A. flavus, A. niger* and *F. oxysporum* f.sp. *lycopersici*. The inhibitory potential of botanical formulations on fungal pathogens has been widely reported (Singh *et al*., 1999; Rajeswari, 2002) [11, 8]. Kumbhar *et al*. (2001) [3] reported that the combined formulations of *Capsicum annuum* (green chilli), *Zingiber officinale* and *Allium sativum* with 5 per cent cyclohexane exhibited better antifungal potential against *A. niger, A. flavus* and *F. oxysporum* under *in vitro* conditions. The results of the present study confirmed that the formulations from chloroform extract of *P. minus* are having high level of stability in all respects. The potential of botanicals in plant disease management is known for a long period and the main demerit of it is; should be prepared afresh and used immediately. Development of new botanical formulation with high storability will answer this problem and will gain importance in the present era of sustainable agriculture.

Table 1: Emulsion stability of different EC formulations

Formulation	Creamy appearance/100 ml measuring cylinder		Sedimentation/100 ml measuring cylinder	
	Below 2 ml	Above 2ml	Below 2 ml	Above 2ml
Polymin 30EC	+	-	+	-
Polymin 40EC	+	-	+	-

+ - Positive
- - Negative

Table 2: Shelf life of Polymin 40EC formulation and its effect on fungal pathogens

Days after inoculation	*A. solani**		*A.flavus**		*A. niger**		*F. oxysporum* f.sp. *lycopersici* *	
	A	B	A	B	A	B	A	B
15	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
30	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
45	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
60	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
75	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
90	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0	0.01 (0.46)	100.0
105	6.94	92.18	7.21	92.38	7.65	91.99	6.56	92.72
130	12.06	86.41	11.50	87.84	11.90	87.54	10.98	87.81
Control	88.76		94.60		95.50		90.05	

CE- Chloroform extract of *P. minus*

A-Diameter of mycelial growth in petridish (mm); B- Per cent reduction over control

*Mean of three replications

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