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Optimization of Dodecanoic Acid oil-in-water microemulsion – Preparation and its Characterization

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ABSTRACT: The objective of the study was to optimize and characterize a dodecanoic acid oil-in-water microemulsion which can be used against Papaya Ring Spot Virus disease. Papaya Ring Spot Virus disease is one of the most destructive diseases which affects papaya production worldwide. The production of bioactive compounds has advanced significantly in recent years which are known for being environmentally safe, harmless to non-target organisms, and exhibiting high development potential. The GC-MS analysis indicated that dodecanoic acid a bioactive compound that was present in the wild genotype of papaya *Vasconcellea candamarcensis* (72.8%) and CO.7 (17%) a cultivated variety of papaya, but absent in the wild types and other cultivated varieties. Dodecanoic acid comes under saturated medium-chain fatty acid groups which are responsible for anti-viral activity in plants. The solubility of dodecanoic acid was analyzed by dissolving dodecanoic acid in ethanol, MCT oil, castor oil, and distilled water. In this study, Dodecanoic acid was encapsulated in medium-chain triglycerides (MCT oil), Tween 80(surfactant), span 20(surfactant), and Glycerol (co-surfactant) as emulsifiers under different concentrations and it is being characterized using FT-IR spectroscopy. These microemulsions prepared will be evaluated against Papaya Ring Spot Virus.

Keywords: Dodecanoic acid, solubility, oil-in-water emulsion, MCT oil, FT-IR spectroscopy.

INTRODUCTION

Papaya Ring Spot Virus was one of the most destructive diseases which was transmitted by aphids in a non-persistent manner (Kalleshwaraswamy *et al*., 2010). Papaya Ring Spot Virus (PRSV) is a member of the genus potyvirus and the family potyviridae which infects papaya (Ashwini *et al*., 2021; Chalak *et al*., 2017) and is transmitted through aphids (Thirugnanavel *et al*., 2015). Apart from the cultivated varieties, some wild-type relatives showed resistance activity against the Papaya Ring Spot Virus Disease. Studies on the assessment of plant essential oils against the aphid species *Myzus persicae* were also done (Pascual-Villalobos *et al.,* 2019). Essential oil compounds (EOCs) are molecules that have well-documented antimicrobial and anti-pest activity (Fernández-Peña *et al.,* 2019). Dodecanoic acid (Lauric acid) a bioactive compound present in the wild genotype of papaya belongs to a saturated fatty acid member of the sub-group of medium-chain fatty acid with a bright white, powdery solid which is insoluble in water and acts as a molecular entity capable of donating a hydron to an acceptor. Numerous studies have found that fatty acids (FAs) affect plant basal resistance to bacterial and fungal pathogens, but there have been few reports on antiviral agents (Zhao *et al.,* 2017). Dodecanoic acid was responsible for the antiviral activity against the groundnut bud necrosis virus (Sangeetha *et al.,* 2020). Dodecanoic acid was considered the most active inhibitor of the virus (Liang *et al.,* 2021) without affecting cell viability and inhibited a late maturation stage in the replicative cycle of Junin virus (Bartolotta *et al.,* 2001). Though these bioactive compounds are fatty acids there is a need that they should be emulsified for further application in plants. Therefore, microemulsion systems havea wide range of technological applications as microemulsion is a thermodynamically stable isotopic liquid mixture of oil, water, surfactant, and co-surfactant (Xavier-Junior *et al.,* 2016). A series of oil in water (O/W) microemulsions were prepared at a constant temperature using the drop-by-drop method with Span80/Tween80 as the composite emulsifying system and Macol-52 as the oil phase (Chen *et al.,* 2020). Thus, microemulsion requires high surfactant levels to provide an interfacial surface to completely microemulsify the compounds and also to provide formulation stability to temperature and storage. The three basic types of microemulsions are direct (oil-in-water), reversed (water-in-oil), and bicontinuous based on thermodynamic stability (Yadhav *et al.*, 2018). In microemulsion systems, where two immiscible phases (water and oil) are present with a surfactant that forms a monolayer at the interface between oil and water. Studies on microemulsions showed that the use of microemulsions as a delivery platform improves the targeted action. The Fourier transform infrared (FT-IR) spectroscopy was used in this study to record the infrared spectrum of absorbance and transmission of the microemulsion.

MATERIALS AND METHODS

Materials. This study was carried out in the Department of Fruit Science and the Department of Nanoscience & Technology, TNAU, Coimbatore to prepare and characterize dodecanoic acid microemulsion. Dodecanoic acid (Lauric acid) purchased from Sigma Aldrich and MCT oil purchased from Smy oils limited (medium-chain triglycerides) was used as the oil phase and tween 80 as a surfactant, span20 as a surfactant, glycerol as co-surfactant, and distilled water was used as the aqueous phase for the preparation of the microemulsions. Magnetic stirrer, magnetic beads (size-25mm), micropipette (100microlitre), beaker, and aluminium foils are used. The oil-in-water microemulsion was prepared in two phases oil phase and aqueous phase, Dodecanoic acid (10mg) + MCT oil (10ml) was used inthe oil phase and tween 80 (surfactant) + span 20 (surfactant) + glycerol at different concentrations are used in the aqueous phase. Using magnetic stirrer microemulsion was prepared, and FT-IR spectroscopy analysis.

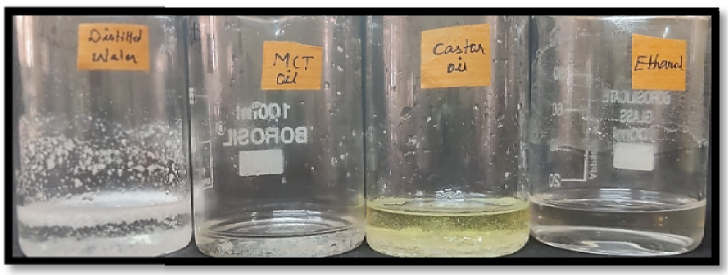

Fig. 1. Solubility check-in dodecanoic acid.

Table 1: Different Concentrations of microemulsion prepared from Dodecanoic acid Four different concentrations of microemulsion were made as LA1, LA2, LA3, and LA4.

Concentrations	Oil-Phase	Time	Aqueous-Phase	Time	Microemulsion	RPM
LA1	Dodecanoic acid (10mg) + MCT oil (10ml)	2 hours	Tween 80 (1ml) + glycerol(100µl)	1 hour	The oil phase is dispersed slowly into the aqueous phase and kept in a magnetic stirrer for 3 hours	600 rpm
LA2	Dodecanoic acid (10mg) + MCT oil (10ml)	2 hours	Tween 80 (0.5ml) + span 20(0.5ml) + glycerol(100µl)	1 hour	The oil phase is dispersed slowly into the aqueous phase and kept in a magnetic stirrer for 3 hours	600 rpm
LA3	Dodecanoic acid (10mg) + MCT oil (10ml)	2 hours	Span20(0.5ml) + tween 80 (0.5ml) + glycerol (100µl)	1 hour	The oil phase is dispersed slowly into the aqueous phase and kept in a magnetic stirrer for 3 hours	600 rpm
LA4	Dodecanoic acid (10mg) + MCT oil (10ml)	2 hours	Span20(1ml) + glycerol(100µl)	1 hour	The oil phase is dispersed slowly into the aqueous phase and kept in a magnetic stirrer for 3 hours	600 rpm

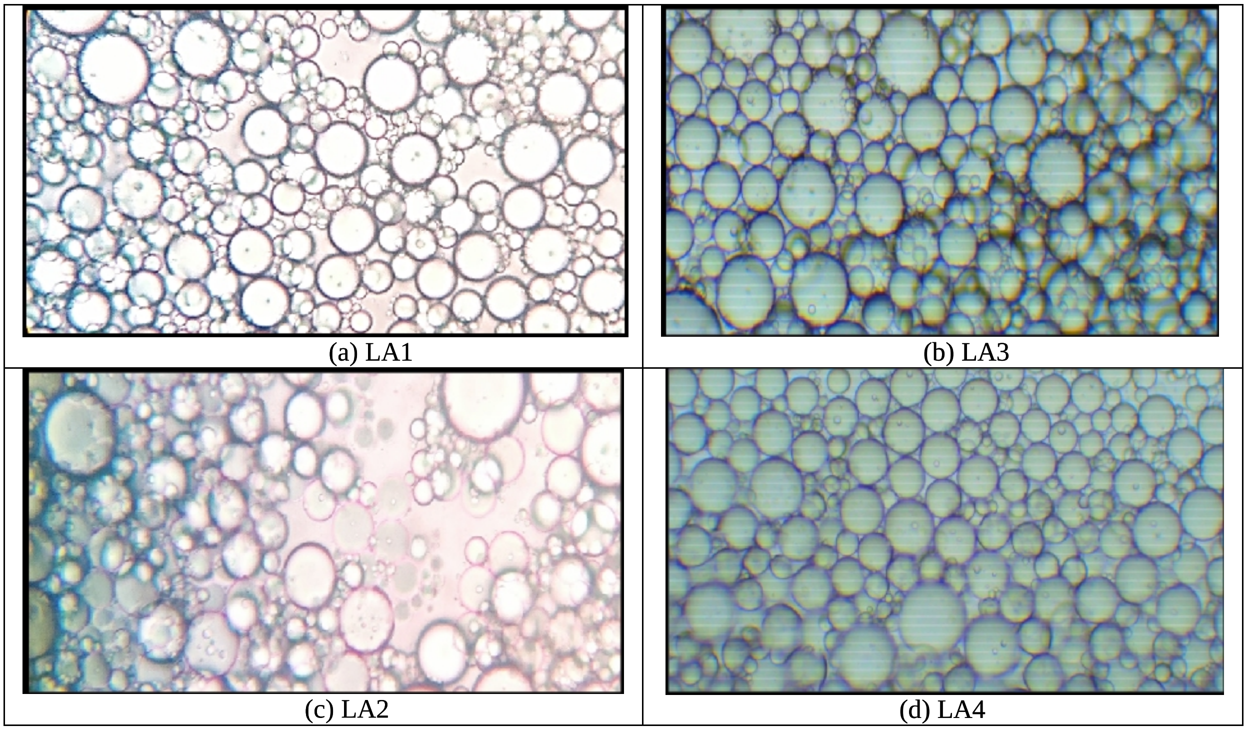

Fig 2: Microscopic image of the microemulsion.

CHARACTERIZATION: FT-IR spectroscopy

FT-IR spectroscopy of model FT/IR-6800 typeA was used to obtain the infrared spectrum and it was recorded between 4000-400 cm^{-1}. The IR spectrum table and chart lists IR spectroscopy frequency ranges, the appearance of the vibration, and absorption for functional groups by frequency range and compound class (Sigma-Aldrich).

Table 2: FT-IR analysis of Dodecanoic acid microemulsion.

Sr. No.	Classification	Group	Wavelength cm^{-1}	Intensity	Mode	Comments
1.	Alcohol	O-H stretching	3342	Strong- broad	Stretching	Intermolecular bonded
2.	Alkane	C-H stretching	2925	Medium	Stretching	
3.	Aldehyde	C=O stretching	1740	Strong	Stretching	
4.	Alkene	C=C stretching	1642	Strong	Stretching	Monosubstituted
5.	Alcohol	O-H Bending	1339	Medium	Bending	

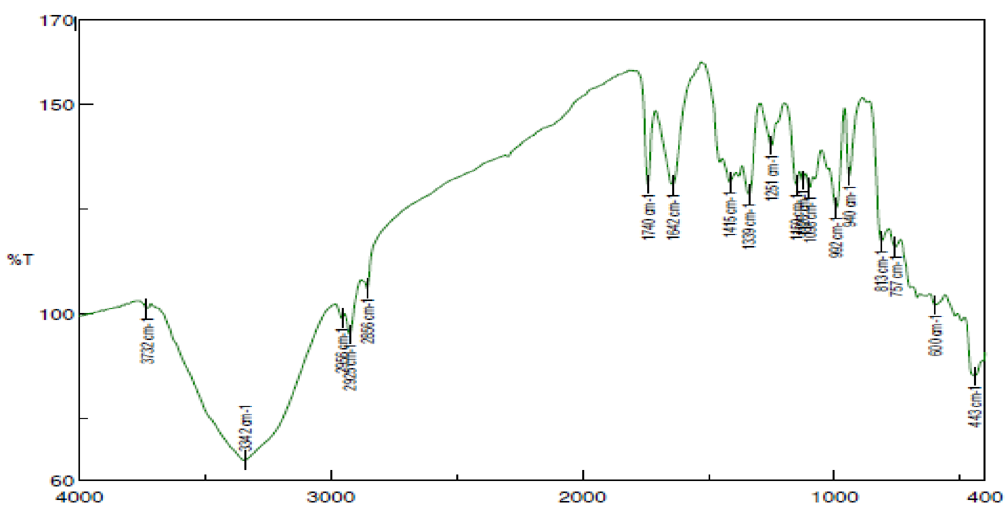

Fig. 3. FT-IR spectrum of Dodecanoic acid microemulsion.

RESULT AND DISCUSSION

Papaya Ring Spot Virus resulted in severe yield loss and much more disease incidence (Premchand *et al*., 2021). Many plants naturally possess bioactive compounds to protect themselves from several diseases by enhancing the resistance capability of the plants and also these bioactive compounds from plants are non-phytotoxic and not harmful to the environment. The GC-MS analysis indicated that dodecanoic acid a bioactive compound that was present in the wild genotype of papayas such as *Vasconcellea candamarcensis* (72.8%) and CO.7 (17%) a cultivated variety of papaya, but absent in wild types and other cultivated varieties (Gohilapriya *et al.,* 2021). Numerous studies have found that fatty acids (FAs) affect plant basal resistance to bacterial and fungal pathogens, but there have been few reports on antiviral agents (Zhao *et al.,* 2017). Dodecanoic acid was considered to be the most active inhibitor of the virus (Liang *et al.,* 2021) without affecting cell viability and inhibited a late maturation stage in the replicative cycle of Junin virus (Bartolotta *et al.,* 2001). For preparing microemulsion the solubility of Dodecanoic acid was checked by dissolving it in ethanol, MCT oil, castor oil, and distilled water and it was dissolved in MCT oil, castor oil, and ethanol rather than distilled water, this shows the solubility of the dodecanoic acid (lauric acid). Dodecanoic acid was encapsulated in medium-chain triglycerides (MCT oil), tween 80, span 20, and glycerol as emulsifiers under different concentrations of oil-in-water microemulsion prepared. The result showed that from different concentrations prepared, dodecanoic acid + MCT oil in the oil phase and distilled water + span 20 + tween 80 + glycerol in the aqueous phase showed greater stability and lesser particle size, and the pH of the microemulsion under different concentrations of LA1, LA2, LA3, LA4 as 7.0, 7.2, 7.3, 7.0 respectively. From FT-IR spectroscopy the infrared spectrum of the functional group of microemulsion was found by frequency range and compound class. The above oil-in-water microemulsion prepared from bio-active compounds can be used against plant viruses, as they are responsible for anti-viral activity in plants due to the presence of ribosome-inactivating protein (Sangeetha *et al.,* 2020). Further confirmation studies are required to study the stability of the preparation as well its efficiency against Papaya Ring Spot Virus (PRSV).

CONCLUSION

This study on oil-in-water microemulsion was prepared from dodecanoic acid a bioactive compound that was present in wild genotypes of papaya, but present in trace amounts in cultivated types CO7 and absent in other cultivated types. These compounds are responsible for the anti-viral property in wild genotypes of papaya and they are also commercially available in the market and can be emulsified as microemulsions and used against papaya Ring spot virus. Though this bioactive compound is responsible for anti-viral properties it may show resistant, repellant, tolerant activity in plants as a defense mechanism and it can also be used against other plant viruses.

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Conflict of Interest. None.

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