Selection of green carrier solvent for the effective creation of bio-inspired liquid formulation and anti-fungal potency evaluation

Aloke Purkait1 · Debasish Rana2 · Argha Banerjee2 · Dipak Kumar Hazra3 · Pabitra Kumar Biswas1 · Ramen Kumar Kole3

Received: 12 April 2022 / Accepted: 4 October 2022 / Published online: 12 October 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract
Existing pesticide formulation solvents generate volatile organic compounds (VOCs), are combustible, and are classed as hazardous air pollutants (HAPs), meaning they are detrimental to users and phytotoxic to crops. Green solvents are required in formulations due to regulations, health, and environmental concerns. In emulsifiable concentration (EC) formulations, the “green chemistry” movement has led to the use of less harmful solvents. After a detailed and comparative fungal growth inhibition assessment, the least harmful carrier solvent among four regularly used organic solvents [dimethyl sulfoxide (DMSO), dimethylformamide (DMF), aromatic hydrocarbon (C9), and methyl oleate] was chosen in this study. We employed methyl oleate (cis-9-Octadecenoic acid methyl ester) as a bio-based green reserver (60%) to create effective bioinspired EC formulations (30%) of Pongamia pinnata L extract utilising emulsifier blends (10 percent) based on the known toxicity order (DMF > DMSO > C9 > methyl oleate). EC1 outperformed the other thirteen formulations (EC1-EC13) in terms of emulsion stability, cold test, accelerated storage stability, flash point, and other metrics, proving its suitability for commercial production. Using four therapeutically appropriate concentrations of agricultural usage, in-vitro fungicidal effects against Alternaria solani and Phytophthora spp. were examined. A. solani (EC50 = 0.08 percent) showed the greatest growth suppression (87.4 percent) at the maximum dosage (1 percent), followed by Phytophthora sp. (71.1 percent) (EC50 = 0.49 percent). The study proved its utility in the production of environmentally acceptable green solvent-based herbal formulations as a long-term crop protection alternative to harmful chemical pesticides.

Keywords Pongamia pinnata extract · Herbal formulation · Emulsifiable concentrate · Emulsion stability · Alternaria solani · Phytophthora sp

Communicated by Erko Stackebrandt.

* Dipak Kumar Hazra
dipakipft@gmail.com

1 Department of Soil Science and Agricultural Chemistry, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, Birbhum, West Bengal 731 236, India
2 Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741 252, India
3 Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741 252, India
Introduction

Chemical fungicides used to protect plants may leave harmful residues that jeopardise human and environmental health, as well as non-target animals (Bhandari et al. 2020; Rani et al. 2020). Barbieri and colleagues (Barbieri et al. 2020). In addition, due to increased crop protection costs as a result of fungicide resistance, the usage of various chemical pesticide classes for plant disease management has been reduced (Gao et al. 2020). As a result, one of the major priorities is to create innovative methods for alternative fungicides for sustainable agriculture (Campos et al. 2018; Lengai et al. 2019).

These days, the use of natural antifungal compounds for plant protection is gaining a lot of traction (Jamiolkowska and Kopacki 2020). *Pongamia pinnata* (L.) seeds contain a wide range of naturally occurring biologically active compounds, including flavonoids, terpenoids, phenols, saponins, alkaloids, and fatty acids (Purkait et al. 2019), as well as antifungal properties (Raja and Sreenivasulu 2016; Shaheen et al. 2017). Bioactive compounds in plant extracts can quickly degrade and volatilize in the field if they are not properly formulated (Borges et al. 2018). As a result, developing commercial formulations to improve the effectiveness of these natural phytochemicals in farmers’ fields may be necessary (Mugao et al. 2020). According to academics, several botanicals have been developed using ordinary aromatic solvents (toluene, xylene, etc.).

These volatile organic solvents, on the other hand, are harmful to users and have been shown to have unacceptable phytotoxicities, as well as significant environmental repercussions due to their non-biodegradability (Donglu et al. 2012). Concerns about food safety and the environment, as well as rising crude oil prices and the detrimental effects of organic solvents, have fueled interest in developing non-petroleum solvents that are readily degradable, reproducible, and non-toxic (Morya et al. 2020). To investigate if vegetable oil derivatives, such as the methyl oleate group of fatty acid methyl esters (FAME), can match the properties and performance of petroleum-based solvents, researchers are studying them (Purkait and Hazra 2019). FAME is a user-friendly, ecologically friendly solvent that is biodegradable and non-toxic, according to the manufacturer (Ping et al. 2016).

The purpose of this study was to investigate the intrinsic toxicity profiles of commonly used organic solvents against *A. solani* and *Phytophthora sp.*, two plant pathogenic fungus. The study’s purpose was to develop herbal antifungal formulations that used Pongamia pinnata seed oil and the least toxic solvent feasible. In-vitro bio-efficacy testing was also conducted against *Alternaria solani* and *Phytophthora spp.*, the pathogens that cause early and late blight in tomatoes and potatoes, resulting in severe damage and high production losses of up to 95.8% (Mugao et al. 2020) and yield losses of up to 79 percent in vegetable crops (Lees et al. 2019).

Methods

Botanicals, chemicals and reagents

Seeds of *Pongamia pinnata* L. (Fabaceae) were taken from a local market and identified. E. Merck India supplied the emulsifiers (Tween 20, Tween 80, nonylphenol ethoxylates, calcium alkylbenzene sulphonates, and dodecylbenzene sulphonate) and carrier solvents [aromatic hydrocarbon (C-9), dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), and methyl oleate]. The reagents were used without further purification. A solution of anhydrous CaCl2 (2.74 mM) and MgCl2.6H2O (0.68 mM) was prepared in double-distilled water (1 L) as standard hard water with a strength of 342 ppm and an electrical conductivity (EC) of 0.69 dSm−1 (CIPAC MT 18, 1995).

Hexane extract preparation

Before being dried in the shade at room temperature, *Pongamia pinnata* seeds were properly cleaned under a moderate flow of tap water to remove dust and other contaminants. Air-dried seeds (1 kg) were ground to powder in a household grinder (Bajaj, Bravo Dlx 500) and extracted twice with hexane (2 + 2) liters in a Soxhlet apparatus for 6 h at 600 C, as reported in our previous research paper (Purkait et al. 2019).
The hexane extract was filtered and dried in a rotary vacuum evaporator (Buchi (R-3), Switzerland) at 40 °C under reduced pressure (370 mbar), yielding (28.37 percent) of the required extract, which was then kept at 4°C for future use. The seed extract was tested for physicochemical characteristics such as refractive index (RI), acid value, iodine value, saponification value, and ester value using conventional techniques (Satish Kumar 2011).

**Toxicity assessment of organic solvents**

**Isolation and maintenance of a pure culture**

Scientists from the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, West Bengal, India, isolated *Phytophthora* sp. and *A. solani* from infected potatoes using a single spore isolation technique and identified them based on colony morphology, morphometric characteristics of acervuli, seta, conidia, and conidiophores, as described by Prittesh (2016). Along the borders of lesions, pathogen-infected potatoes were cut into small pieces (5 mm in diameter). Surface sterilized in a 0.1 percent w/v aqueous mercuric chloride solution, washed five times, streptomycin-dipped, and put on a PDA growth plate. After 8–10 days, mycelial bids were transferred from culture plates to PDA slants and allowed to sporulate.

Scientists from the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Mohanpur, West Bengal, India, isolated *Phytophthora* sp. and *A. solani* from infected potatoes using a single spore isolation technique and identified them based on colony morphology, morphometric characteristics of acervuli, seta, conidia, and conidiophores, as described by Prittesh (2016). Along the borders of lesions, pathogen-infected potatoes were cut into small pieces (5 mm in diameter). Surface sterilized in a 0.1 percent w/v aqueous mercuric chloride solution, washed five times, streptomycin-dipped, and put on a PDA growth plate. After 8–10 days, mycelial bids were transferred from culture plates to PDA slants and allowed to sporulate.

**Bioassay in-vitro**

An in-vitro bioassay was utilized to screen out the toxicities of numerous organic carrier solvents, using the poison food approach (Jang and Kulk, 2018). The content of DMSO, C9, DMF, and methyl oleate in the final test solutions was 1 percent (v/v) according to the Clinical and Laboratory Standards Institute (CLSI) standard guidelines (Hazen 2013). Colony diameters of treatment plates were measured at the point of complete radial growth (9 cm) of each control plate, i.e. 6 days after the incubation period. All treatments and controls were triple-duplicated and incubated at 28.1 °C for the length of the study. The percentage inhibition of mycelial growth was calculated using the following formula (Dutta et al. 2019):

\[
\text{% inhibition of the mycelial growth} = \left(\frac{dc - dt}{dc}\right) \times 100
\]

where dc is the average radial growth (cm) of the test fungus on control plates and dt is the average radial growth (cm) of the test fungus on treatment plates. A difference of p 0.05 was used to indicate a statistically significant difference.

**Preparation of emulsifiable concentrate**

Using the technique discussed in our previous article, multiple emulsifiable concentrate (EC) formulations were developed with varying changes (Purkait et al. 2019). Seed extract (30 percent w/w) was dissolved in the least hazardous solvent (Methyl oleate) (60 percent w/w) in the toxicity assessment of organic solvents (Sect. 2.3). Different emulsifiers in a mix (10%) were added to the extract solution and extensively stirred on a magnetic stirrer at 200 rpm at 50–60°C to prepare EC formulations (Supplementary Fig. 1).

**Physico-chemical characteristics**

As shown in Table 2, *P. pinnata* seed extracts were made in thirteen different EC (30 percent w/w) formulations (EC-1 to EC-13). The physico-chemical characteristics of the formulations were examined in triplicates in accordance with CIPAC and Indian Standard (IS) standards (BIS, 1997).

**Emulsion stability**

The prepared sample (2 mL) was put into a clean, clear beaker (250 mL). Standard hard water was poured over the sample at a rate of 15 to 20 mL min⁻¹ at 32 degrees Celsius to bring the volume up to 100 mL, and the sample was spun continually with a glass rod. The diluted emulsion was transferred to a clean and dry graduated cylinder (100 mL) with a stopper and left undisturbed for one hour to look for any creamy layer on top or deposition on the bottom (CIPAC MT 36.3, 2003).

**Cold test**

The produced sample (50 mL) was placed in a glass container with a stopper in ice-cold water (−10°C). The
container was whirled at short intervals for 1 h to examine for turbidity, an oily coating, or both.

**Flashpoint**

The flashpoints of the developed EC formulations were tested using Abel’s equipment (Scavini, IP0170-110). Each mixture was put in the cup with care and cooked slowly. An external flame was aimed at the cup at regular intervals, and the temperature at which the formulation was lighted was recorded. The flashpoint of the formulation should be over 24.5°C, according to CIPAC MT12 (1995).

**Storage stability evaluation**

In three replicated sets, the formulations were held at elevated temperatures (4, 25, and 54.2°C) for 14 days, equal to a two-year shelf life at ambient temperature (27.2°C) (CIPAC MT46.3, 2000). The EC formulations were visually inspected after fourteen days of storage for phase separation or the formation of a creamy layer.

**pH and specific gravity**

A pH meter calibrated at 25.1°C was used to measure the pH of the generated samples (1 percent aqueous solution) (Systronics, Model 335; Gujarat, India). The specific gravity of the resulting formulations was also evaluated using a calibrated hydrometer (Fisher Scientific, 11–603-4F & 11–603-4G) (CIPAC, 2000).

**Bioassay of an emulsifiable concentrate (30 EC) formulation in vitro**

An in-vitro bioassay based on the suppression of *A. solani* and *Phytophthora sp.* mycelial radial growth was used to test the antifungal activity of the generated EC. Four dosages (0.1, 0.25, 0.5, and 1.0 percent) of the preferred formulation (EC-1) were generated alongside a control in conical flasks.

![Fig. 2](image_url) shows the effect of organic solvents [1 = Methyl oleate; 2 = Aromatic hydrocarbon (C-9); 3 = Dimethyl sulfoxide (DMSO); and 4 = N,N-dimethyl formamide (DMF)] on the growth of two plant pathogenic fungi [A = *A. solani*; and P = *Phytophthora sp.*] at a 1.0% dose.
containing previously sanitized and chilled PDA medium (without formulation). After thorough mixing, 15 ml of medium were poured onto sterilized petri plates (9 cm in diameter).

Aseptic procedures were used to extract five-day-old mycelial discs (7 mm in diameter) and place them individually in the middle of PDA plates. All of the treatments and control plates were triple-duplicated and incubated at 28 degrees Celsius. The colony diameters of the treatment plates were measured at the point of complete radial development (9 cm) of each control plate, which was 6 days after the incubation period. Section 2.3.2 demonstrates how to use the equation to calculate the percentage inhibition of mycelial development (1). (Dutta and colleagues, 2019). The EC50 values of various concentrations were calculated using the logarithm of each concentration and the associated probit value for each inhibition percentage.

## Results

### Toxicity of the solvents

The effects of organic solvents on the growth of A. solani and Phytophthora sp., two plant pathogenic fungi, were investigated (Fig. 2). The solvent DMF inhibited fungal growth the most effectively in A. solani (Fig. 2: A4), whereas DMSO inhibited Phytophthora sp. the most effectively (Fig. 2: P3).

The findings of the fungal growth inhibition are shown in Table 1. DMF (18.2–22.4%) exhibited the highest inhibitory impact on fungal growth, followed by DMSO (16.7–18.7 percent). At doses of 1.0 percent and 0.5 percent DMSO was previously demonstrated to be toxic to Botrytis cinerea (Randhawa 2006; Petruccelli and colleagues 2020).

The least inhibitory effect was seen when methyl oleate (3.3–5.6%) was used as a solvent, followed by C9 (4.7–13.3%) (See Table 1). As a consequence, the solvents'
Development of EC formulations of P. pinnata extract.

The purity and authenticity of the seed extract to be formulated are determined by the optimal values of the physico-chemical properties. Several criteria were investigated, and the findings of the physico-chemical properties like colour (dark brown), odour (pungent), refractive index (1.45), acid value (1.75), iodine value (75), saponification value (182), density (0.92 gmL⁻¹) indicated that the extract was of high quality and might be used formulation purposes. The least dangerous acceptable solvents were P. pinnata seed extract (30%) and methyl oleate (60%) in the creation of Emulsifiable Concentrate (EC) formulations. Different mixes of emulsifiers (such as NP-13, CABS, DBS, Triton–X–100, Tween 20, Tween 80, Span 60, and Span 40) were used to produce the EC formulations (EC1 to EC13) (10 percent). The hydrophilic-lyophilic balance (HLB) value of the blend emulsifiers fulfilled the 10–17 HLB criteria for EC formulation purposes. The generated formulations appeared transparent and light brown due to the natural color of P. pinnata seed extract. The pH (6.4–6.8), specific gravity (0.98), and flash point (66–78°C) of the formulations are all acceptable (Table 2). The EC formulations (EC-3 and EC-10), on the other hand, created unstable emulsions. The formulations (EC-3, EC-6, EC-8, EC-10, EC-11, and EC-13) likewise showed phase separation in the cold test, suggesting that they are inappropriate for further development (Table 3).

Stability of storage

After 14 days of accelerated storage at 4, 25, and 54.2 degrees Celsius, EC-1, EC-5, and EC-9 were found to be stable and passed all physico-chemical tests (Table 2). These compositions produced milky white emulsions with good emulsification blooming capabilities. There was no flocculation or creamy layer separation after a one-hour dilution in standard hard water.

*FAME* methyl oleate group of fatty acid methyl esters (FAME), NP-13 nonylphenol ethoxylates (NP-13), CABS calcium alkyl benzene sulphonates (CABS), DBS dodecyl benzene sulphonate (DBS) [a active matter 60%, b active matter 70%], HLB hydrophilic-lyophilic balance (HLB).

EC1 was chosen as the best formulation because it performed well in a variety of physicochemical tests, including blooming, emulsion stability, re-emulsification, persistent foam, pH, flash point, cold storage, and accelerated storage (Table 2). When put into regular hard water, the chosen mixture flowed well. The selected formulation performed exceptionally well in persistent foam, emulsion stability, and cold tests over a 14-day period at high temperatures. A formulation is regarded successful if it passes all physico-chemical property tests, according to international standards. The surfactant blend with the best HLB value (12.53) for long-term emulsion stability was chosen as EC-1. Modifications in the hydrophile-lipophile balance of the emulsifier system may be required to obtain consistent emulsification in the finished product due to variances in the hydrophile-lipophile balance of the emulsifier system from lot to lot of solvent or batch to batch of pesticidal chemical. The adoption of a paired-emulsifier system makes adjustments more easier. Furthermore, many formulations, including EC-1, are clear, suggesting that the seed oil was evenly dispersed in the solvent.

Unlike other liquid formulations, the solution remained homogeneous and constant throughout application, with no concerns with sedimentation or crystallization. Thakur et al. (2014) employed 10–13 percent emulsifiers to make turmeric oil with a 5–20 percent (w/w) EC. The authors...
developed an EC botanical formulation (30 percent w/w) with just 10% emulsifier blends. Seed extract formulations were also enhanced in terms of content. There was a reduced solvent demand and a smaller number of emulsifiers due to the greater active components, resulting in lesser toxicity.

Results

In-vitro effectiveness against Alternaria solani and Phytophthora sp

In an in-vitro bioassay, the chosen formulation (EC-1 of *P. pinnata* seed extract) was evaluated against two post-harvest plant diseases, *A. solani* and *Phytophthora* sp. The inhibitory impact of the selected formulation (EC-1) on plant pathogen radial development is clearly seen in Figs. 3, 4. The radial development of the fungus was inhibited when the concentration levels of the EC-1 formulation were raised compared with the control.
to the control. Both fungi demonstrated the greatest decrease in fungal growth when given a 1.0 percent dosage of EC-1.

The diameter of the fungal colony in the treated plates was measured at the time of full radial development (9 cm) in each control plate after 6 days of incubation, and the findings are shown in Table 3. The inhibitory efficiency of the EC-1 formulation varies considerably between the two fungus species. At T1 (0.1 percent), the radial growth of A. solani (3.87 cm) was greatly repressed (57%) compared to Phytophthora sp. (6.60 cm), which was only reduced by 26.7 percent (Table 3). As the EC-1 formulation concentrations were raised from 0.1 percent to 1.0 percent, the fungal growth was gradually decreased. At T4 (1.0 percent), the EC-1 formulation suppressed the development of A. solani by 87.4%, but only 71.1 percent in the case of Phytophthora sp (Table 3).

The determined median effective concentration (EC50) value for A. solani was 0.08 percent, and 0.49 percent for Phytophthora sp. in the EC-1 formulation (Table 3). Aqueous, alcoholic, and ethyl acetate crude seed extracts (10–50 percent) of P. pinnata have previously been shown to have antifungal action against A. solani (Kalpashree and Raveesha, 2016; Latha et al. 2009; More and Baig 2013). The acetone leaf extract of P. pinnata suppressed the germination of infestans (L) zoospores by more than 50%. The efficiency of the P. pinnata extract formulation against Phytophthora sp. in the current investigation is much superior to a previous report of only 30% radial growth suppression by 0.05–2.5 percent crude extract (Rani et al. 2015; Sarpong 2016).

### Discussions

Finding an appropriate solvent for the toxicant to be manufactured is the first step in producing an emulsifiable concentration. The emulsifier with the best results in terms of dispersibility and emulsion performance is chosen next. The emulsifier’s solubility and compatibility with the toxicant-solvent system were then determined by storage. When aromatic-type solvents are used, surfactant systems are usually miscible in the formulation. Solvents can affect pesticide penetration through the skin, as well as the composition’s toxicity to the human eye.

As a result, full safety evaluations’ conclusions must be incorporated into labeling. To protect the safety of all people who come into touch with the content, appropriate material safety data sheets should be used. Plant protection compounds’ phytotoxicity is also affected by solvents. Hydrocarbon solvents are often more hazardous to plants than other solvents. Plants are more harmed by higher boiling hydrocarbons than by lighter solvents. Methyl oleate was determined to be the safest organic solvent against both fungi in this investigation, surpassing C-9, DMSO, and DMF (Fig. 2 and Table 1). Solvents with log P values of 1 to 5 are considered to be extremely dangerous to microorganisms (Dyrda et al. 2019). As a result, the lower fungal toxicity of methyl oleate may be explained in part by its higher log P value of 7.45, (Table 4). Methyl oleate (commonly known as biodiesel) is considered non-hazardous and less dangerous than other hazardous solvents such as DMF, according to hazard identification and ecotoxicity ratings (Pan et al. 2020). (See Table 4).

In addition to its reasonable pricing, non-hazardous identity, and low toxicity, methyl oleate has a high flash point, a high boiling range, inflammability, and mid polarity.

| Property                  | Methyl oleate | C-9 | DMSO | DMF |
|---------------------------|---------------|-----|------|-----|
| Viscosity (cp 30°C)       | 3.3–5.2       | 0.88| 2    | 0.79|
| Specific gravity (30°C)   | 0.917         | 0.875| 1.0904| 0.9445|
| Flash point (°C)          | 157–182       | 42  | 89   | 60  |
| Industrial Safety         | Inflammable   | Flammable | Inflammable | Inflammable |
| Hazard(s) identification² | Not considered hazardous | Hazardous | Hazardous | Hazardous |
| log P                     | 7.45          | 3.27| 4.96 | 4.38|
| Eco-toxicity§             | Acute risks to aquatic life is minimal | Very toxic to aquatic life | Harmful to aquatic life | Due to high volatilization, it is low toxic to aquatic life |
| Toxicity                  | Non irritant  | Serious eye irritation | Mild irritant | Severe irritant |
| Price (Rs/Kg)             | 83            | 52  | 125  | 76  |

¹OSHA Hazard Communication Standard 2012; ²WHO 2001

*P* partition coefficient of a given solvent in an equimolar mixture of octanol and water
Because of the low surface tension, interfacial tension and contact angle on the applied surface. Methyl oleate was recognized as a green solvent for the manufacture of herbal fungicidal formulations from *P. pinnata* seed oil.

Seed extract (30%) from the plant *P. pinnata* was converted into a clear and stable emulsifiable concentrate (EC) using biodegradable surfactant blends (10%) that met CIPAC and IS quality criteria (BIS, 1997). Emulsifiers in the LAS category (CABS) are presently a low priority for future study due to their low hazard potential for humans, with the exception of skin and eye irritation and acute inhalation. Exposure to respirable particles is projected to be low, according to information supplied by the sponsoring country. Due to their facile and/or speedy biodegradation and weak bioaccumulation potential, CABS are a low priority for further investigation (Yoneyama et al. 1977). The Office of Pollution Prevention and Toxics (OPPT) evaluated the risks of NP to human health and the environment, concluding that NP’s acute (oral and dermal) toxicity is minimal (EPA 2009c). During the selection of the best and cheapest emulsifier pair, we also examined the cost effectiveness (in Indian rupees) of the tested emulsifiers [CABS (80 kg-1), NP-13 (130 kg-1), SDS (160 kg-1), Tween 20 (160 kg-1), Tween 80 (175 kg-1), Span 60 (150 kg-1) and Span 40 (180 kg-1)]. We ultimately picked two emulsifiers (CBAS and NP-13) that are much less expensive than other investigated surfactants when purchased in bulk quantities, keeping the economic feasibility of the produced formulation in mind.

Under outdoor conditions, plant extract-based green formulations have a hard time preserving the bioactive compounds found in the extracts against rapid degradation and volatilization (Borges et al. 2018). Methyl oleate is a mid-polar fatty acid with good compatibility with *P. pinnata* seed extract and surfactants, as well as a long shelf-life. In recent years, much research has been conducted to create EC formulations using green carrier solvents like as vegetable oils, animal fats, or biodiesels. In EC formulations, green carrier solvents such as methyl ester or glycol diacetate (solvents with low toxicity) are increasingly being used (Zhang et al. 2018). The flash point was about 70 degrees Celsius, which was greater than our previous work (around 50 degrees Celsius), which employed C-9 as a carrier solvent in the EC formulation and provided superior results (Purkait et al. 2019).

Liquid items have a high flash point, making them safer to store, transport, and use (Chin et al. 2012a; 2012b). Methyl oleate also increased oil droplet dispersion by reducing interfacial tension and contact angle on the applied surface. Because of the low surface tension, *P. pinnata* components adsorb well, evenly, and readily on the fungal surface, inhibiting growth effectively (Mukhtar et al. 2019).

The EC-1 formulation concentration (from 0.1 percent to 1.0 percent) significantly increased the suppression of *A. solani* and *Phytophthora* sp. fungal growth. The maximal growth inhibition of two fungi (71.1–87.4%) was observed at the highest concentration level of 1%. Different species of fungus, or even isolates of the same fungal species, may react differently to fungicides. Furthermore, the efficiency of *P. pinnata* seed extracts is highly dependent on the kind, quantity, and stage of maturity of the extracted plant, as well as the pathogen’s mechanism. Antifungal components detected in *P. pinnata* seed extract (17-pentacontene; 2-{[2-methyl-benzo-oxazole-7-yl]-1-H-pyrazole-3-yl]-phenol) may also be responsible for the antibacterial action discovered (Purkait et al. 2020).

### Conclusions

Methyl oleate was determined to be the safest organic solvent against both fungi in this investigation, surpassing C-9, DMSO, and DMF. Seed extract (30%) from the plant *P. pinnata* was converted into a clear and stable emulsifiable concentrate (EC1) using biodegradable surfactant blends (10%) that met CIPAC and IS quality criteria (BIS, 1997). Emulsifiers in the LAS category (CABS) are presently a low priority for future study due to their low hazard potential for humans, with the exception of skin and eye irritation and acute inhalation. Methyl oleate is a mid-polar fatty acid with good compatibility with *P. pinnata* seed extract and surfactants, as well as a long shelf-life. The EC-1 formulation concentration (from 0.1 percent to 1.0 percent) significantly increased the suppression of *A. solani* and *Phytophthora* sp. The maximal growth inhibition of two fungi (71.1–87.4%) was observed at the highest concentration level of 1%. In the production of botanical formulations, the inclusion of a biodegradable carrier solvent (methyl oleate) can make the product more ecologically and user-friendly. The findings might lead to the creation of herbal fungicide formulations as an alternative to harmful synthetic pesticides.

**Acknowledgements** The writers are thankful to Agro-Chemical Formulation Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, West Bengal, India for providing the facilities for this research.

**Author contributions** D.K.H. and R.K.K. wrote the main manuscript text. A.P. and D.R. prepared the figures. A.B. and P.K.B. prepared all tables. All authors reviewed the manuscript.

**Funding** The completion of this research work was done without any financial assistance.
Declarations

Conflicts of interest  The authors declare no conflict of interests.

References

Bhandari G, Atreya K, Scheepers PTJ, Geissen V (2020) Concentration and distribution of pesticide residues in soil. Non-dietary human health risk assessment. Chemosphere 253:1–13. https://doi.org/10.1016/j.chemosphere.2020.126594

BIS Specification (1997) Indian standard methods of test for pesticides and their formulations. IS: First Revision 6940–1982

Bitonto L, Pastore C (2019) Metal hydrated-salts as efficient and BIS Specification (1997) Indian standard methods of test for pesticides and their formulations. IS: First Revision 6940–1982

Borges DF, Lopes EA, Moraes ARF, Soares MS, Visotto LE, Oliveira CR, Valente VMM (2018) Formulation of botanicals for the control of plant-pathogens: a review. Crop Protec 110:135–140. https://doi.org/10.1016/j.cropro.2018.04.003

Campos EVR, Proenca PLF, Oliveira JL, Bakshi M, Abhilash PC, Fraceto LF (2018) Use of botanical insecticides for sustainable agriculture: future perspectives. Ecol Indic. https://doi.org/10.1016/j.ecolind.2018.04.038

Chin CP, Lan CW, Wu HS (2012a) Application of biodiesel as carrier for insecticide emulsifiable concentrate formulation. J Taiwan Inst Chem Eng 43:78–584

Chin CP, Lan CW, Wu HS (2012b) Study on the performance of lambda cyhalothrin microemulsion with biodiesel as an alternative solvent. Ind Eng Chem Res 51(12):4710–4718

CIPAC MT 18 (1995) Preparation of standard waters A and D. In: Dobrat W, Martijn A, editors. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 59–62

CIPAC MT 36.3 (2003) Emulsion stability and re-emulsification in: Dobrat W, Martijn A, eds. CIPAC handbook K. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 137

CIPAC MT 46.3 (2000) Accelerated storage procedure. In: Dobrat W, Martijn A, editors. CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 128

CIPAC MT 75.3. (2000) Determination of pH. In: Dobrat W, Martijn A, editors. CIPAC handbook J. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 131

CIPAC MT12 (1995) Flash point. In: Dobrat W, Martijn A, editors. CIPAC handbook F. Physico-chemical methods for technical and formulated pesticides. Harpenden, England: Collaborative International Pesticides Analytical Council Ltd. 1

Donglu X, Haiyan Y, Puchao L, Guangze C, Min L, Jian K, Fuming C, Min-gzhang C, Min L (2012) Pesticide solvent using jatropha curcas source, and preparation method and application for pesticide solvent. Patent no: CN 102907418A

Dyrdal G, Boniewska-Bernacka E, Man D, Barchewicz K, Slota R (2019) The effect of organic solvents on selected microorganisms and model liposome membrane. Mol Biol Rep 46:3225–3232. https://doi.org/10.1007/s11033-019-04782-y

EPA (2009c) Screening-Level Hazard Characterization. Alkylphenols Category. http://www.epa.gov/chemtrk/hpvis/hazchar/Category_Alkylphenols_Sep2009.pdf

Fernandes CP, Mascarenhas MP, Zibetti FM, Lima BG, Oliveira RPRF, Rocha L, Falcao DQ (2013) HL8 value, an important parameter for the development of essential oil phytopharmaceuticals. Braz J Pharmacog 23:108–114. https://doi.org/10.1590/S0102-695X2012005000127

Gao J, Wang F, Jiang W, Miao J, Wang P, Zhou Z, Liu D (2020) A full evaluation of chiral phenylpyrazole pesticide flupirenone and the metabolites to non-target organism in paddy field. Environ Pollut 264:114808. https://doi.org/10.1016/j.envpol.2020.114808

Hazen KC (2013) Influence of DMSO on antifungal activity during susceptibility testing in vitro. Diagn Microbiol Infect Dis 75(1):60–63. https://doi.org/10.1016/j.diagmicrobio.2012.09.002

Jamiolkowska A, Kopacki M (2020) Natural compounds against plant pests and pathogens. Nat Remed Pest Dis Weed Control. https://doi.org/10.1016/B978-0-12-819304-4.00005-1

Jume BH, Gabris MA, Nadeh HR, Rezania S, Cho J (2020) Biodiesel production from waste cooking oil using a novel heterogeneous catalyst based on graphene oxide doped metal oxide nanoparticles. Renewable Energy 162:2182–2189. https://doi.org/10.1016/j.renene.2020.10.046

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009) Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of pgpr strains and zinnum leaf extract against Alternaria solani. Altern Solani 50(2):85–93. https://doi.org/10.1016/j.biocolution.2009.03.002

Lees AK, Roberts DM, Lynott J, Sullivan L, Brierley JL (2019) Real-Time PCR and LAMP assays for the detection of spores of Alternaria solani and sporangia of Phytophthora infestans to inform disease risk forecasting. Plant Dis 103(12):3172–3180. https://doi.org/10.1094/PPDIS-04-19-0765-RE

Lengai GMW, Mathumii JW, Mbega ER (2019) Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Sci Afric 7:e00239. https://doi.org/10.1016/j.sciaf.2019.e00239

More DR, Baig MMV (2013) Fungitoxic properties of Pongamia pinnata (L) Pierre extracts against pathogenic fungi. Int J Adv Bio-technol Res 4(4):560–567

Morya S, Amoah AEDD, Snaebjornsson SO (2020) Microorganisms for sustainable environment and health. Food poisoning hazards and their consequences over food safety. 383–400. Elsevier.

Mugao LG, Muturi PW, Gichimu BM, Njoroge EK (2020) In-vitro control of Phytophthora infestans and Alternaria solani using crude extracts and essential oils from selected plants. Intern J Agron 2020:1–10. https://doi.org/10.1155/2020/8845692

Mukhtar NAM, Abd Rashid A, Hagos Ftwi Y, Noor MM, Kadiramg K, Rizalman M, Adam AA (2019) The influence of formulation ratio and emulsifying settings on tri-fuel (diesel–ethanol–biodiesel) emulsion properties. Energies 12(9):1708. https://doi.org/10.3390/en12091708

Okumura Y, Koyama J, Takaku H, Satoh H (2001) Influence of organic solvents on the growth of marine microalgae. Archiv Environ Contam Toxicol 41(2):123–128. https://doi.org/10.1007/s002440010229

OSHA Hazard Communication Standard (2012) https://www.osha.gov/dsg/hazcom/ (Accessed on 2021)

Pan L, Chang P, Jin J, Yang Q, Xing F (2020) Dimethyformamide inhibits fungal growth and aflatoxin b1 biosynthesis in Aspergillus flavus by down-regulating glucose metabolism and amino acid biosynthesis. Toxins 12(11):683. https://doi.org/10.3390/toxins12110683

Petrucelli V, Brasili E, Varone L, Valletta A, Pasqua G (2020) Anti-fungal activity of dimethyl sulfoxide against Botrytis cinerea and other...
phytotoxicity on tomato and lettuce plants. Plant Biosys Intern J
Deal All Aspect Plant Biol 154(4):455–462. https://doi.org/10.1080/11263504.2020.1779846
Ping H, Hongmei Y, Donghui W, Jianhua L, Guohua Z, Jianfeng Y,
Chunlin W, Baojiang D (2016) Dimethyl dichloroviny phosphate
emulsifiable concentrate and preparation thereof. Patent no:
CN 105613566
Pubchem, NIH, US, National Library of Medicine, National central
for biotechnology information. https://pubchem.ncbi.nlm.nih.gov/
(Accessed on 2021)
Purkait A, Biswas S, Saha S, Hazra DK, Roy K, Biswas PK, Ghosh
SK, Kole RK (2019) Formulation of plant based insecticides, their
bio-efficacy evaluation and chemical characterization. Crop Prot
125:104907. https://doi.org/10.1016/j.croprot.2019.104907
Raja RR, Sreenivasulu M (2016) *Pongamia Pinnata*—Phytotherapeutic
review. World J Pharm Res 5(4):505–511
Randhawa MA (2006) The effect of dimethyl sulfoxide (DMSO) on
the growth of dermatophytes. Nippon Ishinkin Gakkai Zasshi
47(4):313–318. https://doi.org/10.3314/jjmm.47.313
Rani A, Shukla G, Sing R, Kumar A, Girdharwal V (2015) Antifungal
activity of plant extracts against *Phytophthora in festans*. Intern
J Sci Res 4(8):666–668
Rani A, Singh R, Kumar P, Shukla G, Singh C (2017) Ecofriendly man-
agement of late blight of potato caused by *Phytophthora infestans*
(L.). Intern J Pharm Res Technol 1:21–27
Rani L, Thapa K, Kanoja N, Sharma N, Singh Sukhbir G, Ajmer S,
Srivastav AL, Kaushal J (2020) An extensive review on the con-
sequences of chemical pesticides on human health and environment.
J Clean Prod. https://doi.org/10.1016/j.jclepro.2020.124657
Sarpong MT (2016) In vitro evaluation of the effect of selected plant
extracts on the *Phytophthora* fungus causing disease in MD2 vari-
ety of pineapple in the central region of Ghana. EC Microbiol
4(1):623–632
Satish Kumar BN (2011) Pharmacological studies of *Pongamia pinnata*
(Linn.) Pierre. Int J Pharm Sci Res 9:12–19
Shaheen I, Parveen S, Parveen Z (2017) Evaluation of *Pongamia pin-
ndata* products against the *Sclerotium rolfsii* extracted from chick-
pea. Adv Crop Sci Tech 5:291. https://doi.org/10.4172/2329-8863.
1000291
Thakur LK, Roy S, Prajapati R, Singh MK, Raza SK, Mangave BD,
Singh A, Jha S (2014) Development and evaluation of environ-
ment & user friendly turmeric oil emulsifiable concentrate (EC)
formulations for postharvest quality and life in rose CV. Poison
Int J Recent Sci Res 5:178–185
WHO (2001) Concise International Chemical Assessment Document
31https://www.who.int/ipcs/publications/cicad/en/cicad31.pdf?
ua=1 (Accessed on 2021).
Yoneyama M, Masubuchi M, Oishi S, Takahashi O, Ikawa M, Yoshida
S, Oishi H, Mikuriya H, Yuzawa K, Hiraga K (1977) Subacute
toxicity of linear alkylbenzene sulfonate. Ann. Rep. Tokyo
Metrop. Res. Lab. Public Health 28:73–84 (in Japanese); cited
in: IPCS (1996); Environmental Health Criteria 169: Linear
Alkylbenzene Sulfonates (LAS) and Related Compounds. WHO,
Geneva, Switzerland.
Zhang XP, Jing TF, Zhang DX, Luo J, Li BX, Liu F (2018) Assess-
ment of ethylene glycol diacetate as an alternative carrier for use
in agrochemical emulsifiable concentrate formulation. Ecotoxicol
Environ Saf 163:349–355
Publisher’s Note Springer Nature remains neutral with regard to
jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under
a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article
is solely governed by the terms of such publishing agreement and
applicable law.