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# Evaluation of mustard oil emulsion against root-knot nematode, *Meloidogyne incognita*, and wilt fungus *Fusarium oxysporum*

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**Abstract**
Mustard oil is known to exhibit activity against nematodes and pathogens due to the presence of Ally Isothiocyanate. Since the past decade, the use of plant-based pesticides is becoming more popular and a lot of research is being done worldwide on the use of plant metabolites for protecting crops against various biotic factors. In tomatoes, a disease complex involving root-knot nematode, *Meloidogyne incognita*, and wilt fungus, *Fusarium oxysporum* is known to cause severe loss in yield. This study was conducted to develop an emulsion of mustard oil and evaluate it against *M. incognita* and *F. oxysporum*. Since Mustard oil has low water solubility, it was stabilized into emulsion using surfactants (Tween 80 & Span 80). Mustard oil emulsion thus obtained was tested against the *Meloidogyne incognita* and *Fusarium oxysporum*. At 4000ppm concentration, Mustard oil emulsion caused 100% mortality of *M. incognita* infective juveniles and completely inhibited the hatching of nematode eggs. In the Potato Dextrose Agar medium amended with the emulsion, a clear zone of *F. oxysporum* growth inhibition was observed indicating the fungicidal activity of mustard oil emulsion. Based on the present study it is concluded that Mustard oil emulsion has outstanding effects against *Meloidogyne* incognita and *Fusarium oxysporum*. Additional field studies using the developed emulsion will be a step forward in precision agriculture.

**Keywords:** Mustard oil, Allyl Isothiocyanate, emulsion, *Meloidogyne incognita*, *Fusarium oxysporum*, precision agriculture

## Introduction
Plant-parasitic nematodes are the most ubiquitous organisms and are considered important pathogens for all agricultural crops. Root-knot (*Meloidogyne spp.*) and cyst nematodes (*Globodera rostochiensis*) are the most economically important pest nematodes for crop failure (Briar, Wichman, & Reddy, 2016) [2]. Apart from that, they can act as carriers of pathogenic bacteria, fungi, and viruses which damage the plants. Nematodes assist or accelerate diseases caused by fungi and bacteria, and also in some instances break the resistance in cultivars (M. W. Khan, 1993) [11]. Root-knot nematodes (*Meloidogyne incognita*) are considered one of the most significant biotic restraints in agriculture (Haq, Mukhtar, Haq, & Khalid, 2022) [6]. They are found in a wide variety of plant species including vegetables i.e. tomatoes, coffee, bananas, papaya, cucumbers, guavas, and forestry severely affecting yields and productivity (del Prado Vera, Soto, & Hernndez, 2001) [5].

*Fusarium spp*. cause severe plant damage and crop loss, because of the synergistic interaction of plant parasitic nematodes (PPN) with *Fusarium spp.* (M. R. Khan, Fischer, Egan, & Doohan, 2006) [10] When root-knot nematodes invade plants, it worsens the wilt symptoms and increases the death rate of plants. The root exudates of nematode-infected plants stimulate *Fusarium* propagules and conceal actinomycetes, which are responsible for controlling the wilt fungus in plants. (Bernard, Egnin, Bonsi, & control, 2017) [1]. This disease conjunction, produced by root-knot nematodes and *Fusarium spp.* poses a serious risk to many crops. (Wang, McSorley, & Kokalis-Burelle, 2006) [15].

Many methods exist to control the plant parasitic nematodes and soil-borne pathogens like soil solarization, cover cropping and soil fumigation. With regard to soil fumigation, chemical fumigants are frequently employed to manage nematodes and soil-borne diseases but they are also hazardous to soil and human health. Therefore, biological approaches have enormous

potential in sustainable agriculture. Plant-based fumigation is an eco-friendly management technique for PPN control. A natural plant defensive mechanism called glucosinolates (GSLs), which are produced by the Brassicaceae family of plants, is used in biofumigation. There are around 200 distinct GSLs reported, most of them are produced from glucose and amino acids containing sulfur and nitrogen. The enzyme myrosinase, which is contained in myrosin cells, hydrolyzes GSLs stored in vacuoles after plant tissue has been damaged which turns isothiocyanates (ITCs). Sustainable agricultural techniques utilize this chemical reaction by raising GSL-rich cover crops and incorporating to promote ITC production for biofumigation.

Based on the fact that Mustard oil is rich in AITC, this study was carried out with the objective of evaluating mustard oil for the control of nematodes and wilt fungus. Since mustard oil is insoluble in water it cannot be used directly in the field to combat nematodes and soil-borne diseases. Hence an emulsion containing mustard oil which is soluble in water was developed and evaluated for its nematicidal and fungicidal properties.

## Materials and Methods

### Materials

Mustard oil was procured from the local market and an emulsion was prepared using Span 80 Tween 80 and Paraffin oil. All the chemicals were obtained from the Isochem Laboratory, Kochi, India. Demineralized water was used for standardizing the emulsion. The above materials are used without any further purification for all experiments. For the egg hatching test of Root-knot nematode, the egg masses were obtained from the pure culture maintained at the Department of Nematology. *Fusarium oxysporum* pure culture was obtained from the Centre for Agricultural Nanotechnology, Tamil Nadu Agricultural University, Coimbatore.

### Formulation of mustard oil emulsion

The emulsion made with mustard oil was standardized in a 1:4 ratio using demineralized water. The internal phase was made up of oil attached with surfactants Tween 80 and Span 80, surrounded by water phase. Later Paraffin oil was added to the oil phase in order to reduce the HLB value of the oil, followed by the addition of Span 80. The oil phase was mixed with the water phase slowly, by stirring for an hour at 350 rpm. Then, the formulation was sonicated for 5 minutes at 40% amplitude (20 KHz) in a probe ultrasonicator. The below-mentioned equation was used to calculate Hydrophilic-Lipophilic balance (HLB) value

$$\frac{HLB_{resultant}: \text{Required } HLB - HLB_{Span\ 80}}{HLB_{Tween\ 80} - HLB_{Span\ 80}} \times 100$$

### Bio efficacy of Mustard Oil emulsion against the eggs and juveniles of *Meloidogyne incognita* under in-vitro conditions

### Egg hatching test

The mustard oil emulsion was diluted in water at different concentrations (1000, 2000, 3000, 4000, 5000ppm) and transferred to 5 cm petri plates, and three egg masses were placed in each Petri dish. The experiment was carried out in a completely randomized design with three replications. The hatching percentage was estimated for 3 days at an interval of 24, 48, and 72 hours.

### Juvenile mortality test

The juvenile mortality test was performed on freshly hatched *M. incognita* juveniles. Various concentrations of Mustard oil emulsion (1000, 2000, 3000, 4000, 5000 ppm) were taken in a Petri plate with a diameter of 5 cm, and then 100 juveniles were introduced. Data was obtained from three replications for each treatment conducted in a completely randomized design. The juvenile mortality was estimated for 3 days at an interval of 24, 48, and 72 hours.

### Antifungal activity efficacy studies of Mustard oil emulsion under in-vitro conditions against *Fusarium oxysporum*

Different concentration of mustard oil emulsion (100, 500, 1000, 2000, 3000) ppm was evaluated against *Fusarium oxysporum* using the food poison technique (Janssen, Scheffer, & Svendsen, 1987) [8]. One ml diluted formulation was added to 15 ml Potato Dextrose Agar and allowed for solidification. A 5mm fungal disc from the margins of the actively growing culture of *F. oxysporum* was placed at the centre of the petri plates. The radial growth of the fungus was measured after 7 days of incubation under room conditions. The percentage of inhibition in the radial growth of the *Fusarium oxysporum* was calculated.

### Statistical analysis

The data obtained was processed through statistical differences of the parameters and was analysed using Analysis of variance (ANOVA) and Completely Randomized Design CRD with SPSS and R software.

## Results and Discussion

### Bio-efficacy of mustard oil emulsion against Root-knot nematode, *M. incognita* under *in-vitro* condition

To evaluate the effectiveness of mustard oil emulsion in preventing the hatching of *M. incognita* eggs, various concentrations of the emulsion were tested. Out of the concentrations tested, Mustard oil emulsion at 4000 ppm and 5000 ppm totally suppressed the egg hatching when compared to the control. When the *M. incognita* egg masses were exposed to a concentration of 1000 ppm of mustard oil emulsion, there was 57.87% inhibition in egg hatching compared to control, at 72 hours after treatment (Table 1). Though egg hatching was seen at 1000 ppm concentration, all hatched juveniles from the eggs were found dead. The eggs exposed to AITC were observed through an inverted microscope and compared with unexposed eggs. It could be observed that in unexposed eggs there was normal development and first stage juvenile were present within the eggs. But in eggs exposed to AITC there was no development seen within the egg and first stage juveniles were not formed.

To evaluate the effectiveness of mustard oil emulsion in Juvenile mortality of M. incognita eggs, various concentrations of the emulsion were tested. Out of the concentrations tested, 100% juvenile mortality was seen in Mustard oil emulsion at 4000 ppm and 5000 ppm concentration within 72 hours (Table 2). When the *M. incognita* juveniles were exposed to a concentration of 1000 ppm of mustard oil emulsion, there was 75% mortality observed after 72 hours when compared to control. Similar to the treated eggs, the infectious juveniles exposed to mustard oil emulsion were found dead and with internal body parts

that were malformed, which must be the result of the interaction between AITC and nematode. The present study was in confirmation with the report of Paul Dhalin and Johannes Hallmann 2020 [4], where significant reduction in root knot nematode *Meloidogyne hapla* was observed due to AITC (Dahlin & Hallmann, 2020) [4]. Proposed mechanisms of AITC include inhibition of cell metastasis by suppression of MAPK pathway (Lai *et al*., 2014) [12], causing DNA damage by $O^{2-}$ formation (Murata, Yamashita, Inoue, & Kawanishi, 2000) [14], inducing glutathione S-transferase (GST) expression in Caenorhabditis elegans (Hasegawa, Miwa, Tsutsumiuchi, & Miwa, 2010) [7], influencing protein structures by disrupting disulfide bonds in bacteria (Kawakishi & Kaneko, 1987) [9], and killing fungal cells by inducing an oxidative stress response as in the case of *Alternaria brassicicola* (Calmes *et al*., 2015) [3].

**Anti-fungal activity of Mustard oil emulsion against *Fusarium oxysporum***

The antifungal activity of Mustard oil emulsion was assessed by poison food technique by adding different concentrations of emulsion to Potato Dextrose Agar media. The radial growth of the fungus *Fusarium oxysporum* in Petri plates was measured after 7 days. Among the treatments Mustard oil emulsion at 5000 ppm concentration completely inhibited the growth of fungus (Table 3, Fig 1). There was 100% reduction in colony growth at 5000 ppm. The lower concentrations also had inhibitory effect (44.44% to 73.64%) on the fungus, *F. oxysporum*. The outcome of the current study showed that AITC causes aberrant hyphal development and mycelial structure shrinkage. Similar changes were reported in the *F. solani* hyphal structure when exposed to AITC (Li *et al*., 2020) [13].

**Table 1:** Effect of Mustard oil emulsion on hatching of *M. incognita* eggs under in-vitro conditions

| Treatments | Number of hatched juveniles (Mean of three replications) | | | | | |
|---|---|---|---|---|---|---|
| | 24h | | 48 h | | 72h | |
| | Mean and transformed value | Percent decrease over control | Mean and transformed value | Percent decrease over control | Mean and transformed value | Percent decrease over control |
| T1 – 1000ppm Mustard oil emulsion | $78^{d}$ (8.84) | 35 | $95^{d}$ (9.77) | 55.81 | $115^{d}$ (10.75) | 57.87 |
| T2 –2000ppm Mustard oil emulsion | $66^{c}$ (8.18) | 45 | $86^{c}$ (9.30) | 60 | $103^{c}$ (10.19) | 62.27 |
| T3 – 3000ppm Mustard oil emulsion | $2^{b}$ (1.25) | 98.33 | $2^{b}$ (1.25) | 99.06 | $2^{b}$ (1.25) | 99.26 |
| T4 – 4000ppm Mustard oil emulsion | $0^{a}$ (0.70) | 100 | $0^{a}$ (0.70) | 100 | $0^{a}$ (0.70) | 100 |
| T5 – 5000ppm Mustard oil emulsion | $0^{a}$ (0.70) | 100 | $0^{a}$ (0.70) | 100 | $0^{a}$ (0.70) | 100 |
| Control | $120^{e}$ (10.98) | | $215^{e}$ (14.67) | | $273^{e}$ (16.54) | |
| S.Ed | 0.29 | | 0.30 | | 2.37 | |
| CD (p= 0.01%) | 0.99 | | 1.007 | | `0.986 | |

Figures in parentheses are square root transformed values.

**Table 2:** Effect of Mustard oil emulsion on mortality of *M. incognita* juveniles under in-vitro conditions

| Treatments | Mean and transformed value of dead infective juveniles (Mean of three replications) | | | | | |
|---|---|---|---|---|---|---|
| | 24h | Percent mortality (%) | 48h | Percent mortality (%) | 72h | Percent mortality (%) |
| T1 – 1000ppm Mustard oil emulsion | $54^{d}$ (7.38) | 54 | $63^{d}$ (7.87) | 63 | $75^{d}$ (8.64) | 75 |
| T2 – 2000ppm Mustard oil emulsion | $60^{c}$ (7.75) | 60 | $76^{c}$ (8.53) | 76 | $82^{c}$ (9.05) | 82 |
| T3 – 3000ppm Mustard oil emulsion | $67^{b}$ (8.20) | 67 | $82^{b}$ (9.22) | 82 | $90^{b}$ (9.50) | 90 |
| T4 – 4000ppm Mustard oil emulsion | $78^{ab}$ (8.86) | 78 | $94^{a}$ (9.54) | 94 | $100^{a}$ (9.983) | 100 |
| T5 – 5000ppm Mustard oil emulsion | $83^{a}$ (9.14) | 83 | $99^{a}$ (9.779) | 99 | $100^{a}$ (9.99) | 100 |
| Control | $2^{e}$ (1.25) | 2 | $5^{e}$ (2.24) | 5 | $10^{e}$ (3.16) | 10 |
| S.Ed | 0.31 | | 0.13 | | 0.08 | |
| CD (p=0.01) | 1.06 | | 0.402 | | 0.23 | |

Figures in parentheses are square root transformed values.

**Table 3:** Anti-fungal activity of Mustard oil nano-emulsion against *Fusarium oxysporum* under in-vitro conditions

| Treatments | Radial growth of fungus in (cm) Mean of three replications | Percent decrease over control |
|---|---|---|
| T1 – 1000ppm Mustard oil emulsion | $4.5^{d}$ (2.12) | 44.44 |
| T2 – 2000ppm Mustard oil emulsion | 4.0c (2.0) | 50.62 |
| T3 – 3000ppm Mustard oil emulsion | $2.6^{b}$ (1.60) | 67.90 |
| T4 – 4000ppm Mustard oil emulsion | $2.2^{b}$ (1.49) | 73.84 |
| T5 – 5000ppm Mustard oil emulsion | $1.5^{a}$ (1.22) | 81.49 |
| Control | $8.1^{e}$ (2.85) | |
| CD (0.01) | 0.15 | |
| SE(d) | 0.04 | |

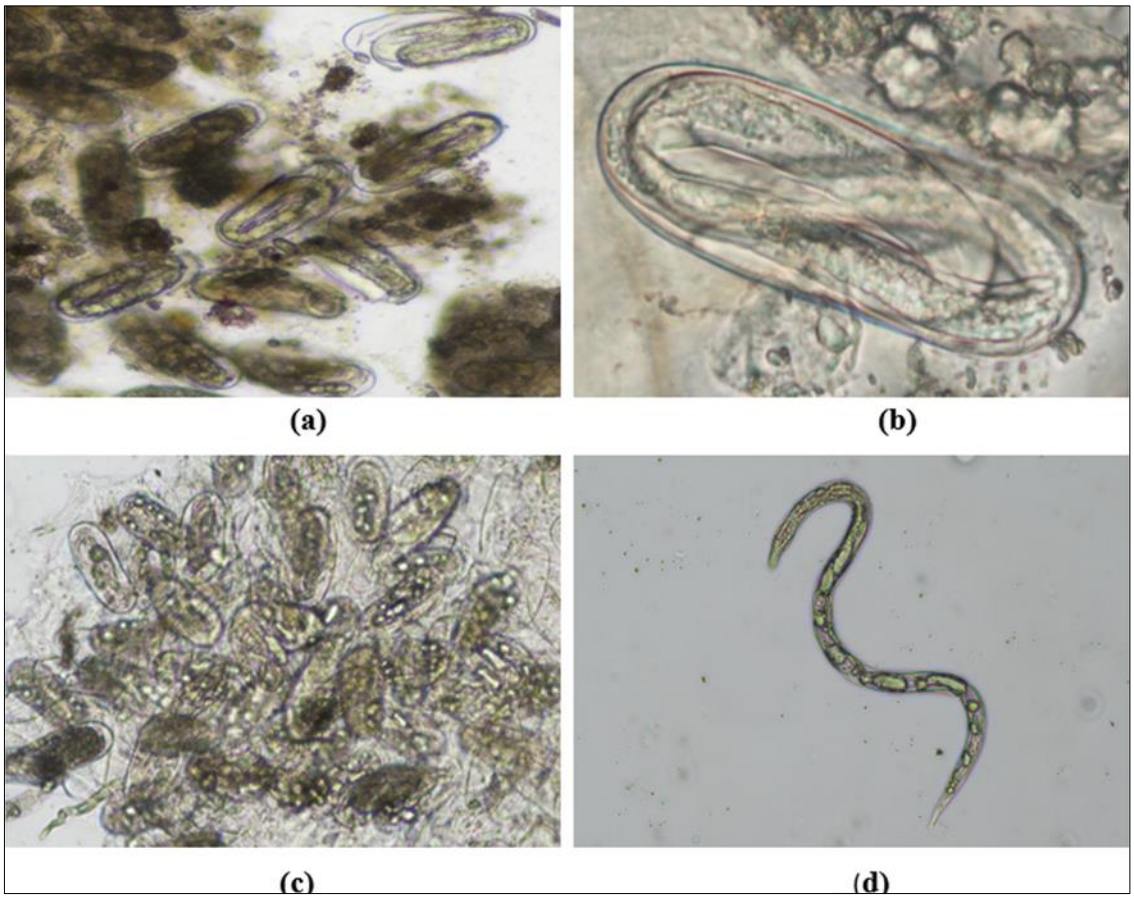


**Fig 1:** Images taken from the Inverted Research Microscope ECLIPSE Ti2, (a) & (b) untreated eggs, (c) & (d) Mustard oil emulsion treated eggs and juveniles

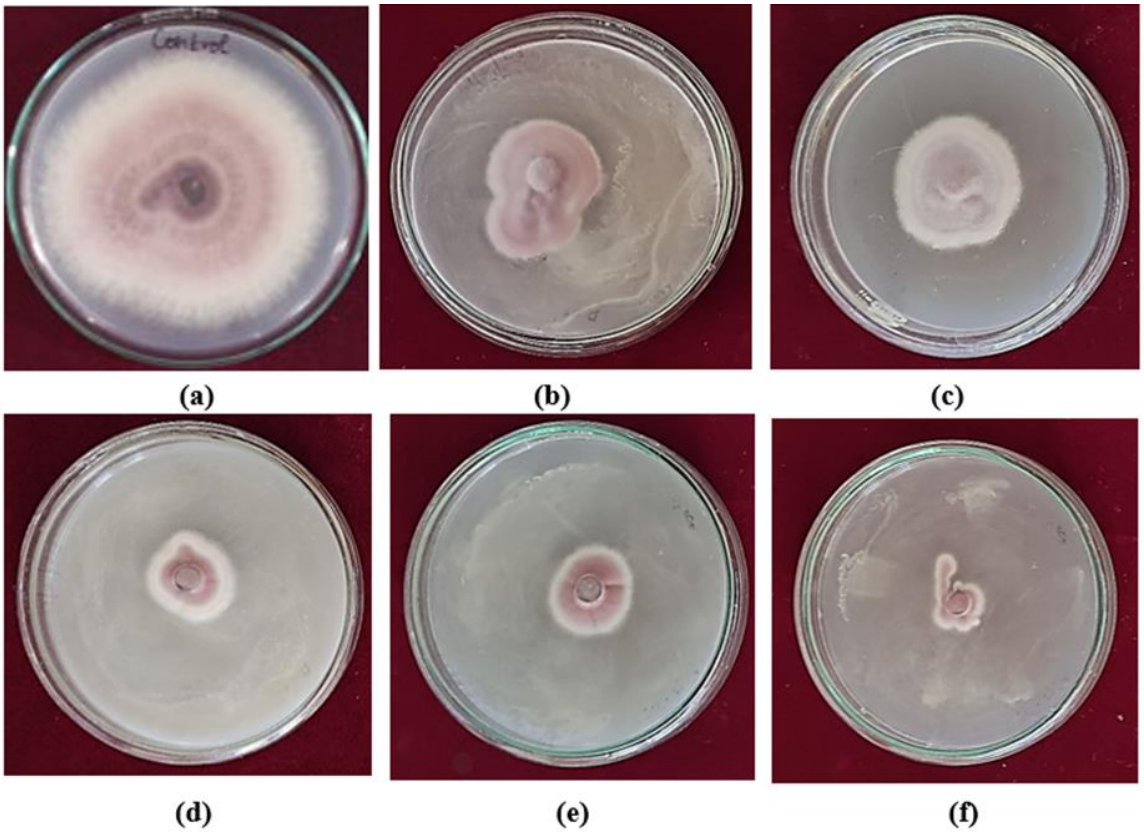


**Fig 2:** Radial Growth of Fusarium oxysporum (a) control (b) Mustard oil emulsion 1000ppm, (c) 2000 ppm, (d) 3000 ppm, (e)4000ppm, (f) 5000 ppm analysized through poison food technique.

## Conclusion

In the present study, an emulsion of mustard oil containing AITC was formulated. The developed product was found to significantly inhibit hatching of *M. incognita* eggs and cause mortality of infective juveniles. It was also found effective against root wilt fungus *F. oxysporum*. Since AITC has the capacity to substitute chemical fumigants because of its high effectiveness, low hazard, and low molecular weight, the product developed has to be further evaluated for its efficacy under field conditions.

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