C. R. Biologies 340 (2017) 214–225

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Plant biology and pathology/Biologie et pathologie végétales

Developing eco-friendly biofungicide for the management of major seed borne diseases of rice and assessing their physical stability and storage life

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ARTICLE INFO

Article history:
Received 22 September 2016
Accepted after revision 1st March 2017
Available online 4 April 2017

Keywords:
Rice seeds
Plant oils
EC formulation
Biofungicide
Seed treatment

ABSTRACT

Three plant oils (*Cymbopogon citratus*, *Cymbopogon martini*, and *Pelargonium graveolens*) were developed as EC formulations and tested for their physical stabilities. EC formulations (10EC, 20EC and 30EC) of *C. citratus*, *C. martini* and *P. graveolens* had emulsion stability, spontaneity property, heat and cold stability. EC formulated plant oils were screened against the major seed borne fungi of rice such as *Curvularia lunata*, *Fusarium moniliforme*, *Bipolaris oryzae*, and *Sarocladium oryzae*. The level of inhibition varied among the concentrations of EC formulations. Among the three EC formulations, that of *C. citratus* oil 30EC recorded 100% inhibition on the mycelial growth of test pathogens. In the blotter paper method, rice seeds treated with a formulation of *C. citratus* oil 30EC controlled the infection of *C. lunata, F. moniliforme, B. oryzae* and *S. oryzae* in rice seed to the tune of 66.0%, 60.4%, 66.0% and 69.1%, respectively. Seed soaking with formulation of *C. citratus* oil 30EC showed the highest percentage of normal seedlings, the lowest number of abnormal seedling and fresh ungerminated seeds when tested with the roll-towel method. Seed soaking with 30EC formulation of *C. citratus* oil increased seed germination, shoot length, root length and vigour of rice seedlings when tested with the plastic tray method. Transmission of pathogens from seed to seedling was reduced significantly by the 30EC formulation of *C. citratus* oil when tested with the plastic pot method. The effect of the storage life of the 30EC formulation of *C. citratus* oil showed that it had retained their antifungal effect till the end of the incubation period (120 days), and is able to inhibit the mycelial growth of all test pathogens to the 100% level.

1. Introduction

Rice (*Oryza sativa* L.) is one among the foremost important cereal crop among the world and is a primary food crop for half of the world's population. Globally, 158.8 million ha of rice is grown with production of 738 million tonnes [1], among them 90% of rice grown and consumed in Asia alone. As of today, rice production represents 30% of the world cereal production. It has doubled in the last 30 years, due to the introduction of new varieties [2]. Asian countries produced 90% of rice, with China and India being the major producers. The other

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http://dx.doi.org/10.1016/j.crvi.2017.03.001
1631-0691/

major producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, and Japan [3].

Using quality seed is an important input for the successful production of any crop. Good-quality seed should possess major characteristics such as viability, purity, free from varietal mixtures and free from seed borne pathogen infection and high yielding potential [4]. In spite of the above-mentioned qualities required for fighting seed infection, seed-borne diseases may provoke the introduction of new pathogens, both quantitative and qualitative crop losses and permanent contamination of soil [5].

A total of 153 seed-borne pathogens have been detected from rice seeds so far, of which 18% are of quarantine importance, 65% are native isolates and 17% are storage pathogens [6]. The predominant seed-borne fungal pathogens of rice that cause infection and proliferate in the field are *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *Sarocladium oryzae*, *Alternaria alternate*, and so on [7].

So far, chemical and biological methods have been used to alleviate and control the seed-borne diseases of rice. Chemical methods, such as spraying fungicide [8] or using fumigants like methyl bromides [9], are harmful to the environment [10]. Hence, recent efforts have focused on developing environmentally safe, long-lasting and effective control measures like using plant-derived essential oils for the control of plant diseases. Various plant essential oils have been reported to have antifungal activities with no side-effects on humans and animals [11]. Essential oils derived from medicinal plants contain non-phytotoxic compounds that are potentially effective against several microorganisms including many fungal pathogens [12] such as several seed-borne, soil-borne and foliar pathogens [13–15]. Fungicidal and bactericidal properties of essential oils have long been studied and characterized [16,17] for the presence of active compounds as alkaloids, phenols, flavonoids, monoterpenes, sesquiterpenes, and isoprenoids [18].

In our previous similar works, we isolated and identified four major seed-borne pathogens such as *C. lunata*, *F. moniliforme*, *B. oryzae* and *S. oryzae*, and have evaluated six plant essential oils, *viz.* (*Cymbopogon citratus*, *C. nardus*, *C. martinii*, *Eucalyptus globulus*, *Ocimum sanctum* and *Pelargonium graveolens*) against these seed-borne pathogens *in vitro*. Among the six essential oils, oils of *C. citratus*, *C. martinii* and *P. graveolens* were selected for further studies based on their efficacy in controlling the seed-borne pathogens of rice [19]. Hence, the present studies were carried out with the following objectives, *viz.* (i) to develop a suitable EC formulation of the selected essential oils and test them for their physical stability under storage at room temperature (ii), assessing the efficacy of different concentrations of EC formulation of essential oils on major seed-borne fungi, on seed germination, seedling vigour (blotter and roll-towel methods) and seed-to-seedling transmission (Plastic pots and tray).

2. Materials and methods

2.1. Efficacy of seed treatment with the selected essential oils against seed-borne fungi

2.1.1. Seed treatment

For each rice cultivar, seeds were collected from our experimental farm and the seed lots were divided into the required number of sub-lots (based on number of treatments) of 50 g each. Eight sub-lots were treated with a range of concentration of selected essential oil (based on an *in vitro* assay) along with chemical control and untreated control (sterile water), respectively. For seed treatment, the soaking method of Gangopadhyay and Kapoor [20] was followed. The efficacy of the essential oils against seed-borne fungi was evaluated based on the standard blotter method [21] and the seed germination test [22]. The treatment schedule followed is mentioned below (T_1 – *Cymbopogon citratus* @0.1%, T_2 – *Cymbopogon martinii* @0.1%, T_3 – *Pelargonium graveolens* @0.1%, T_4 – Thiram @2 g/kg of seed, T_5 – untreated control).

2.2. Efficacy of seed treatment on seed-borne fungi (seed health testing)

2.2.1. Standard blotter method

The standard blotter method International Seed Testing Association [22] was used for assessing the germination percentage of rice seeds. For each seed sample, hundred seeds were taken randomly for each cultivar and 25 seeds per plate were placed in a Petri plate 9 cm in diameter containing three well-moistened blotters and incubated at room temperature. Three replicates were maintained for each treatment. After 7 days of incubation, the germination percentage was recorded by using the formula suggested by Ibiam et al. [23].

$$\mathrm{MPG} = \left(\frac{N_1 - N_2}{N_1}\right) \times 100$$

where MPG is the mean percentage germination, N_1 is the number of treated seeds placed, N_2 is the number of ungerminated seeds.

2.3. Efficacy of seed treatment with the selected essential oils on the growth of rice seedlings by the roll-towel method

For each treatment, 200 seeds (ADT 45 and White Ponni) were tested in three replicates of 50 seeds each, using the between-paper (BP) method including fungicide-treated and -untreated (sterile water) control. After 7 and 14 days of incubation, the seedlings were evaluated for normal, abnormal seedlings and fresh ungerminated seeds according to the International Rules for Seed Testing [22] and expressed in percentage.

2.4. Formulation of essential oil as emulsifiable concentrate

Based on the effective concentration of the selected essential oil from the above studies, further studies were carried out to standardize the oil formulations in order to evaluate their efficacy against seed-borne pathogens. The

materials used for the formulations were solvent, emulsifier, and stabilizer. [24]. The solvent cyclohexanone, the emulsifier Unitox 60® and the stabilizer epichlorohydrin were used for the preparation of various oil formulations. The EC formulations were prepared by mixing a given quantity of plant oils – 10 ml (10EC), 20 ml (20EC), 30 ml (30EC) – 70 ml of emulsifier, 1 ml of stabilizer, the remaining part being filled with an organic solvent. The pH of the EC formulations was maintained at pH 6.6 [25]. The prepared emulsifiable concentrates were tested for physical stability.

2.5. Physical stability evaluations of EC formulations of essential oils

2.5.1. Emulsion stability test

About 2 ml of each EC formulation were taken, and 100 ml of standard hard water (SHW) with an electrical conductivity of 0.5 mhos and hardness equivalent to 342 ppm of $CaCO_3$, was added slowly using a dropping funnel under continuous stirring. The milky white emulsion formed was transferred into a 100-ml measuring cylinder and kept undisturbed for 1 h [25].

2.5.2. Spontaneity test

The EC formulations were added dropwise slowly in a beaker containing 200 ml of water, and the spontaneity property (occurrence of a milky white colour) was observed while mixing [25].

2.5.3. Test for heat and cold stability

Heat and cold stability tests were conducted as per the method described by Meenakshisundaram [25] using water of varying hardness such as distilled water (DW) with an electrical conductivity (EC) of 0.05 mS, hard water (HW) with an EC of 3 mS and Siruvani water (SW) with an EC of 0.057 mS.

This should result in the absence of formation of turbidity or of any solid or oily matter. When EC formulations are treated with hard water/tap water, there should not be any flocculation, turbidity, solid or oily matter.

2.6. Effect of EC formulation of the selected essential oils on the mycelial growth of seed-borne fungi

The three different concentrations (10, 20 and 30EC) of EC formulations of selected oil preparations were screened under laboratory conditions against major seed borne fungi. The concentration of individual formulations (@0.1%) were mixed with sterile PDA medium and plated in respective concentrations. The mycelial disc of the respective seed-borne fungi was inoculated under aseptic condition in the concerned treatments separately and incubated at room temperature ($28 \pm 2\,^{\circ}C$). The radial growth of the pathogen in each treatment was recorded when the control plate has covered the entire plate; the percentage of reduction of the mycelial growth *versus* the control was calculated [25].

2.7. Efficacy of a seed treatment with EC formulations of C. citratus oil on rice seed infection (ADT 45 and White Ponni) by seed-borne fungi

The incidences of different fungi associated with the seeds were assessed by following the standard blotter method [22]. For each seed sample, 200 seeds were taken for each cultivar and treated with respective concentrations of EC formulated oils @0.1% along with chemical control (thiram @2 g/kg of seed) and untreated control (sterile water), respectively. Twenty-five seeds per plate were placed in a Petri plate 9 cm in diameter containing three well-moistened blotters. The fungus observed was identified by using the method of Burnett and Hunter [26]. The total numbers of seeds infected by specific seed-borne fungi were scored to determine the percentage of seed infection. The potency or efficacy of the essential oils was determined as % fungal recovery, using the formula proposed by Ibiam et al. [23].

2.8. Efficacy of the seed treatment with EC formulations of C. citratus oil on the growth of rice seedling

For each treatment, 200 seeds were tested in three replicates of 50 seeds each, using the between-paper (BP) method including fungicide-treated and -untreated (sterile water) control as described in earlier methods. After 7 and 14 days of incubation, the seedlings were evaluated for normal, abnormal seedlings and fresh ungerminated seeds according to the International Rules for Seed Testing [22] and expressed in percentage.

2.9. Efficacy of EC formulations of C. citratus oil on the seedling vigour of rice (ADT 45 and White Ponni)

Seedling vigour tests were conducted in sand with a ratio of 1:1:1 (red soil: FYM: sand). The seeds were treated with respective concentrations of essential oils (@0.1%) along with chemical control (thiram @2 g/kg of seed) and untreated control (sterile water), respectively. The treated seeds were sown in plastic trays (25×20 cm). One hundred seeds were selected at random from a total of 400 seeds/sample and sown on sand in each plastic tray in four lines (25 seeds/line). After seven days of incubation, the germination percentage was recorded by using the formula suggested by Ibiam et al. [23].

After 20 days, shoot and root lengths were measured. Fifteen seedlings from each tray were randomly selected for the measurement of shoot and root length. The seedling vigour was determined following the formula of Baki and Andersen [27] as shown below:

$$\text{vigor index} = (\text{mean root length} + \text{mean shoot length}) \times \text{percentage of seed germination.}$$

2.10. Efficacy of EC formulations of C. citratus oil on seed-to-seedling transmission of seed borne fungi

A fraction of treated and non-treated seeds from each one of the two rice cultivars ADT 45 and White Ponni were

sown in sterilized soil in plastic pots in three replicates of 50 seeds. After 14 days, the seedlings were assayed as per the procedure mentioned by Nguefack et al. [28] for the recovery of *C. lunata*, *B. oryzae*, *F. moniliforme* and *S. oryzae*. Two sections (S1 and S2) from each seedling were plated for recovery of the fungi in question. The sections were described as follows: S1 (portion of the seedling from the mesocotyl), S2 (portion of the seedling on either side of the coleoptile tip). The experiment was repeated thrice and the effect on the seed-to-seedling transmission was calculated for each fungus as the per cent difference of recovery between the non-treated and treated seeds.

2.11. Storage life of 30EC formulation of C. citratus oil

The 30EC formulation of *C. citratus* oil was stored at room temperature ($28 \pm 2\ ^{\circ}C$) for different periods *viz.*, 30, 60, 90 and 120 days. Samples were drawn at monthly intervals up to 4 months. Bioassays on the inhibition of growth of test fungi were carried out up to 120 days [29]. The method was described in earlier studies.

2.12. Statistical analysis

All the experiments were of completely randomized design (CRD) and repeated twice. Data were subjected to analyses of variance, and treatment means were compared by an appropriate Duncan's multiple-range test ($P < 0.05$). The IRRISTAT package version 92-1, developed by the International Rice Research Institute Biometrics Unit, Philippines, was used for analysis [30].

3. Results

3.1. Effect of selected essential oils on rice seed germination by blotter method

Rice seeds (ADT 45 and White Ponni) were treated with essential oils to assess the germination percentage. The essential oils of *C. citratus*, *C. martinii* and *P. graveolens* as well as fungicide exhibited a stimulatory effect on seed germination. Among the three essential oils tested, lemongrass (*C. citratus*) oil was found to be the best for rice seedlings. Seed soaking with lemongrass oil (85.6% and 83.6%) followed by Thiram (84.6% and 84.4%) and palmarosa oil (83.0% and 82.4%) increased the seed germination percentage of rice variety ADT 45 and White Ponni (Fig. 1). However, the control recorded the lowest germination of 63.0 and 61.6 per cent respectively.

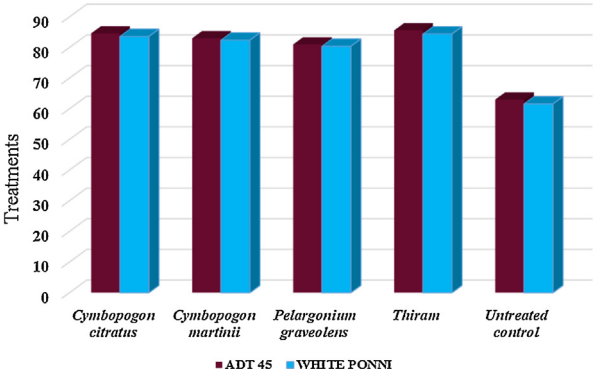

Fig. 1. Efficacy of the selected essential oils on rice seed germination by the blotter method for two rice varieties.

3.2. Efficacy of seed treatment with selected essential oils on the growth of rice seedlings by the roll-towel method

Two rice varieties (ADT 45 and White Ponni) were treated with three selected essential oils for assessing the normal, abnormal seedlings and fresh ungerminated seeds (Table 1). The results of the present study revealed that seed soaking (ADT 45 and White Ponni) with lemongrass oil (*C. citratus*) showed the highest percentage of normal seedlings (93.6% and 92.6%) and the lowest percentage of abnormal seedlings (3.4% and 3.8%) and fresh ungerminated seeds (3.0% and 3.6%) respectively. This was followed by seeds treated with thiram, palmarosa and geranium oil in decreasing order.

3.3. Physical stability evaluation of EC formulations of selected essential oils

3.3.1. Emulsion stability test

All the EC formulations of *C. citratus*, *C. martinii* and *P. graveolens* (10EC, 20EC and 30EC) had the emulsion stability, since the volume of milky white cream formed at the top and sediments at the bottom were not exceeded beyond 2 ml.

3.3.2. Spontaneity test

The prepared EC formulations (10EC, 20EC and 30EC) of three selected oils were mixed easily with water, produc-

Table 1
Efficacy of seed treatment with the selected essential oils on the growth of rice seedlings (roll-towel method).

Treatment	ADT 45			White Ponni		
	Normal seedlings (%)	Abnormal seedlings (%)	Fresh ungerminated seeds (%)	Normal seedlings (%)	Abnormal seedlings (%)	Fresh ungerminated seeds (%)
T_1 – *Cymbopogon citratus*	93.6 a	3.4 a	3.0 a	92.6 a	3.8 a	3.6 a
T_2 – *Cymbopogon martinii*	90.6 c	5.8 c	3.6 b	91.2 b	5.4 c	3.4 b
T_3 – *Pelargonium graveolens*	91.0 c	5.4 c	3.6 b	90.6 c	6.0 d	3.4 b
T_4 – Thiram	92.2 b	4.6 b	3.2 a	91.6 b	4.8 b	3.6 a
T_5 – Untreated control (sterile water)	72.6 d	15.6 d	11.8 c	70.8 d	16.6 e	12.6 c

Values are mean of three replications.
In a column means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

ing a milky white emulsion instantaneously. Hence, the three EC formulations of three essential oils have the spontaneity property.

3.3.3. Test for heat stability

The result of this test clearly showed that there was no formation of turbidity, solid or oily matter. Thus it proved that it was stable even under hot conditions.

3.3.4. Test for cold stability

The cold stability test proved that there was no formation of turbidity, solid or oily matter. The results depicted that it was stable even under cold condition.

3.4. Effect of EC formulations of plant oils on the mycelial growth of C. lunata, F. moniliforme, H. oryzae, *and* S. oryzae

The data depicted in Table 2 revealed that the level of mycelial growth inhibition varied between the concentrations of EC formulations. Among the three EC formulations, the 30EC formulation of *C. citratus* oil recorded complete inhibition on the mycelial growth of all the test pathogens, compare to control. This was followed by 20EC (96.0, 95.0, 94.2 and 93.0% respectively) and 10EC (93.3, 93.0, 90.0 and 91.0% respectively) formulation.

Generally, all EC formulations have the capacity to inhibit the growth of test pathogens. Of the EC formulations tested, the 30EC formulation of *C. martinii* oil recorded the higher inhibition on the mycelial growth (90.7, 89.8, 86.4 and 90.5% mm, respectively) of the test pathogen over the control (Table 2). This was followed by 20EC (85.8, 85.8, 82.7 and 88.7% respectively) and 10EC (80.0, 81.5, 78.9 and 83.5% respectively) formulations.

The EC formulations of *P. graveolens* oils at 10, 20 and 30EC were tried *in vitro* at 0.1% concentration against the mycelial growth of the rice seed-borne pathogen. The 30EC formulations were significantly effective with 89.8%, 90.7%, 86.6%, and 94.1% of mycelial growth inhibition of *C. lunata*, *F. moniliforme*, *B. oryzae* and *S. oryzae* compared to control, followed by 20EC (84.7, 88.0, 79.5 and 91.0%, respectively) and 10 EC (80.0, 81.9, 75.3 and 85.8%, respectively) formulations (Table 2).

The results revealed that all the three EC formulated (10, 20 and 30EC) selected oils (*C. citratus*, *C. martinii* and *P. graveolens*) have the capacity to inhibit the mycelial growth of four seed-borne pathogens. Among these, the EC formulated *C. citratus* oil showed more than 90% of inhibition on the mycelial growth of *C. lunata, F. moniliforme*, *B. oryzae* and *S. oryzae*. Hence, the EC formulated oil of *C. citratus* alone was taken for the subsequent experiment.

3.5. Efficacy of seed treatment with EC formulation of C. citratus *oil on rice seed infection by* C. lunata, F. moniliforme, B. oryzae *and* S. oryzae *(blotter paper method)*

Rice seeds were treated with EC formulated plant oils against test pathogens. Among the EC formulations tested, the 30EC formulation of *C. citratus* oil controlled the level of infection of *C. lunata*, *F. moniliforme*, *B. oryzae*, and *S. oryzae* in rice seeds by 66.0%, 60.4%, 66.0% and 69.1%, respectively, for the rice variety of ADT45, whereas the values were 49.0%, 54.3%, 62.7% and 66.1%, respectively, for the White Ponni variety (Table 3). This was followed by seeds that were treated with Thiram. The effect of 20 and 10EC formulations were considerably lower than that of the 30EC formulations and of the fungicide. The control was recorded as having the highest percentage of seed infection.

3.6. Efficacy of seed treatment with EC formulations of C. citratus *oil on the growth of rice seedlings (roll-towel method)*

Two rice varieties (ADT 45 and White Ponni) were treated with three concentrations of the EC formulation (10, 20 and 30EC) of *C. citratus* oil for assessing the normal, abnormal seedlings and fresh ungerminated seeds following the roll-towel method. The results revealed that seed soaking (ADT 45 and White Ponni) with the 30EC formulation of *C. citratus* oil showed the highest percentage of normal seedlings (92.6% and 90.8%), the lowest number of abnormal seedlings (4.4% and 5.5%), and of fresh ungerminated seeds (3.0% and 3.8%), respectively. This was on par with the chemical treatment (Table 4). However, the untreated control recorded the lowest percentage of normal seedlings (72.6%); the highest number of abnormal (15.6%) and fresh ungerminated seeds (11.8%) for the variety ADT45, whereas White Ponni recorded 70.8%, 16.6% and 12.6%, respectively (Table 4).

3.7. Efficacy of the EC formulations of Cymbopogon citratus *oil on the seedling vigour of rice (sand using the plastic tray method)*

The data depicted in Table 5 indicate that rice seeds (ADT 45 and White Ponni) treated with three different

Table 2
Effect of the EC formulations of plant oils on the radial growth of rice seed-borne pathogens.

EC formulations of geranium oil	Radial growth (mm)											
	Cymbopogon citratus				*Cymbopogon martinii*				*Pelargonium graveolens*			
	C. lunata	*F. moniliforme*	*B. oryzae*	*S. oryzae*	*C. lunata*	*F. moniliforme*	*B. oryzae*	*S. oryzae*	*C. lunata*	*F. moniliforme*	*B. oryzae*	*S. oryzae*
10 EC	6. 0 c	6.6 c	9.0 c	8.0 c	17.8 c	16.4 c	19.0 c	14.6 c	17.2 c	16.0 c	22.2 c	12.6 c
20 EC	3.2 b	4.2 b	5.2 b	6.0 b	12.6 b	12.6 b	15.6 b	10.0 b	13.6 b	10.6 b	18.4 b	8.4 b
30 EC	0.0 a	0.0 a	0.0 a	0.0 a	8.2 a	9.0 a	12.2 a	8.4 a	9.0 a	8.2 a	12.0 a	5.2 a
Control	89.0 d	88.6 d	90.0 d	89.0 d	89.0 d	88.6 d	90.0 d	89.0 d	89.0 d	88.6 d	90.0 d	89.0 d

Values are means of three replications.
In a column, means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

Table 3
Efficacy of the seed treatment with EC formulations of *Cymbopogon citratus* oil on rice seed infection of seed-borne pathogens (blotter paper method).

EC formulation of lemongrass oil	Seed infection (%)															
	ADT 45								White Ponni							
	C. lunata	Per cent reduction over control	*F. moniliforme*	Per cent reduction over control	*B. oryzae*	Per cent reduction over control	*S. Oryzae*	Per cent reduction over control	*C. lunata*	Per cent reduction over control	*F. moniliforme*	Per cent reduction over control	*B. oryzae*	Per cent reduction over control	*S. Oryzae*	Per cent reduction over control
10EC	7.2 c	28.0	5.2 c	45.8	6.6 c	37.7	6.9 b	37.2	7.6 b	20.8	6.3 c	31.5	6.8 c	33.3	8.4 c	32.2
20EC	6.2 b	34.8	4.2 b	56.2	4.8 b	54.7	7.2 c	41.9	7.0 b	27.1	5.8 b	36.9	5.2 b	49.0	7.6 b	38.8
30EC	3.4 a	66.0	3.8 a	60.4	3.6 a	66.0	3.4 a	69.1	4.9 a	49.0	4.2 a	54.3	3.8 a	62.7	4.2 a	66.1
Thiram	3.6 a	64.0	4.0 a	58.3	3.8 a	64.2	3.6 a	67.3	5.0 a	48.0	4.3 a	53.3	4.0 a	60.7	4.4 a	64.5
Control	10.0 d	–	9.6 d	–	10.6 d	–	11.0 d	–	9.6 c	–	9.2 d	–	10.2 d	–	12.4 d	–

Values are means of three replications.
In a column means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

Table 4
Efficacy of seed treatment with EC formulations of *Cymbopogon citratus* oil on the growth of rice seedlings (roll-towel method).

Sl. No	EC formulations	Conc. of oils (%)	ADT 45			White Ponni		
			Normal seedlings (%)	Abnormal seedlings (%)	Fresh ungerminated seeds (%)	Normal seedlings (%)	Abnormal seedlings (%)	Fresh ungerminated seeds (%)
1	10	0.1	87.4 b	7.8 b	4.8 b	84.8 c	8.4 b	6.8 b
2	20	0.1	88.8 b	6.6 c	4.6 b	87.4 b	7.2 c	5.4 c
3	30	0.1	92.6 a	4.4 a	3.0 a	90.8 a	5.5 a	3.8 a
4	Thiram	–	92.2a	4.6 a	3.2 a	90.4 a	5.3 a	3.6 a
5	Untreated control	–	72.6 c	15.6 d	11.8 c	70.8 d	16.6 d	12.6 d

Values are means of three replications.
In a column, means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

concentrations of the EC formulation of *C. citratus* oil differed significantly with respect to germination percentage, shoot length, root length, and vigour index. Among the three EC formulations tested, seed soaking (ADT 45) with 30EC formulation of *C. citratus* oil was found to be the best for rice seedlings. Seed soaking with 30EC formulation of *C. citratus* oil increased seed germination, shoot length, root length, and vigour of rice seedling (90.6%, 5.6 cm, 17.6 cm and 2101.9, respectively) and of the variety White Ponni (89.0%, 4.6 cm, 16.6 cm, and 1886.8, respectively). This was on par with the chemical treatment. The highest vigour index was recorded for the 30EC formulation of *C. citratus* oil (Table 5). The other two EC formulations (20 and 10EC) were in decreasing order of efficacy.

3.8. Effect of seed treatment with EC formulations of C. citratus oil on rice seed-to-seedling transmission of C. lunata, F. moniliforme, B. oryzae and S. oryzae, (sand using plastic pots)

The variety ADT45, transmission of *C. lunata* from seed to seedling was reduced significantly by 91.8, 88.6 and 82.1% with the 30, 20 and 10EC formulation of *C. citratus* oil formulations, respectively and 90.2% with Thiram. Seed treatment with 30, 20 and 10EC formulation of *C. citratus* oil reduced the seed-to-seedling transmission of *F. moniliforme* by 91.8, 81.8, and 79.0%, respectively. These reductions were significantly higher than the 90.9% reduction obtained with Thiram. The seed treatment with three concentrations (30, 20 and 10EC) of EC formulation of *C. citratus* oil and Thiram reduced significantly the seed-seedling transmission of *B. oryzae* by 90.2, 86.4, 80.6% and 90.2%, respectively. The transmission of *S. oryzae* from seed to seedlings was reduced significantly by 90.9, 83.8 and 79.7% with the 30EC, 20EC and 10EC formulations of *C. citratus* oil, respectively and 86.8% with Thiram (Table 6).

The variety White Ponni transmission of *C. lunata* from seed to seedling was reduced significantly by 86.4, 79.1 and 72.9% with the 30, 20 and 10EC formulations of *C. citratus*, respectively, and by 83.3% with Thiram. Seed treatment with the 30, 20 and 10EC formulations of *C. citratus* oil reduced the seed-to-seedling transmission of *F. moniliforme* by 90.1, 81.3 and 73.5%, respectively. These reductions were significantly higher than the 87.2% reduction obtained with Thiram. Seed treatment with three concentrations (30, 20 and 10EC) of the EC formulation of *C. citratus* oil and Thiram reduced significantly the seed-to-seedling transmission of *B. oryzae* by 89.5, 83.8, 77.4, and 89.5%, respectively. The transmission of *S. oryzae* from seed to seedlings was reduced significantly by 87.9, 82.4 and 78.7% with the 30EC, 20EC and 10EC formulation of oil, respectively, and 85.1% with Thiram (Table 6).

Based on the results obtained from the previous experiments, one concludes that the rice variety ADT 45 performs better when compare to the variety White Ponni. Hence, the variety ADT 45 alone was retained for further studies.

3.9. Effect of the storage life of the 30 EC formulation of C. citratus oil for different periods at room temperature on the mycelial growth of C. lunata, F. moniliforme, B. oryzae, and S. oryzae

The 30 EC formulation of *C. citratus* oil was tested for its stability at room temperature ($28 \pm 2\,^{\circ}C$) for different

Table 5
Efficacy of EC formulations of *Cymbopogon citratus* oil on seedling vigour of rice (sand using plastic tray method).

EC formulations	ADT 45				White Ponni			
	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour index	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
10EC	80.4 c	2.9 c	13.0 c	1278.3	79.6 c	2.0 c	11.6 c	1082.5
20EC	86.0 b	3.6 b	15.3 b	1625.4	85.6 b	3.3 b	15.3 b	1592.1
30EC	90.6 a	5.6 a	17.6 a	2101.9	89.0 a	4.6 a	16.6 a	1886.8
Thiram	89.6 a	5.3 a	17.6 a	2051.8	88.6 a	4.3 a	16.9 a	1878.3
Control	63.2 d	1.0 d	9.3 d	650.9	65.0 d	1.3 d	10.0 d	734.5

Values are means of three replications.
In a column, means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

Table 6
Efficacy of seed treatment with EC formulations of *Cymbopogon citratus* oil on rice seed-to-seedling transmission of *Curvularia lunata, Fusarium moniliforme, Bipolaris oryzae*, and *Sarocladium oryzae* (sand using plastic pots).

EC formulation of lemongrass oil	Seed-seedling transmission (%)															
	ADT 45								White Ponni							
	C. lunata	Per cent reduction over control	*F. moniliforme*	Per cent reduction over control	*B. oryzae*	Per cent reduction over control	*S. oryzae*	Per cent reduction over control	*C. lunata*	Per cent reduction over control	*F. moniliforme*	Per cent reduction over control	*B. oryzae*	Per cent reduction over control	*S. Oryzae*	Per cent reduction over control
10EC	2.2 c	82.1	2.3 b	79.0	2.0 b	80.6	2.0 c	79.7	2.6 c	72.9	2.7 c	73.5	2.8 c	77.4	2.3 c	78.7
20EC	1.4 ab	88.6	2.0 b	81.8	1.3 a	86.8	1.6 b	83.8	2.0 b	79.1	1.9 b	81.3	2.0 b	83.8	1.9 ab	82.4
30EC	1.0 a	91.8	0.9 a	91.8	1.0 a	90.2	0.9 a	90.9	1.3 a	86.4	1.0 a	90.1	1.3 a	89.5	1.3 a	87.9
Thiram	1.2 a	90.2	1.0 a	90.9	1.0 a	90.2	1.3 a	86.8	1.6 a	83.3	1.3 a	87.2	1.3 a	89.5	1.6 a	85.1
Control	12.3 d	–	11.0 c	–	10.3 c	–	9.9 d	–	9.6 d	–	10.2 d	–	12.4 d	–	10.8 d	–

Values are means of three replications.
In a column, means followed by a common letter are not significantly different at the 5% ($P = 0.05$) level by DMRT.

Table 7
Effect of the storage life of the 30EC formulation of *Cymbopogon citratus* oil for different periods at room temperature on the radial growth of *Curvularia lunata*, *Fusarium moniliforme*, *Bipolaris oryzae*, and *Sarocladium oryzae*.

Days after storage	Radial growth (mm)							
	C. lunata	Per cent reduction over control	*F. moniliforme*	Per cent reduction over control	*B. oryzae*	Per cent reduction over control	*S. oryzae*	Per cent reduction over control
30	0.0 a	100	0.0 a	100	0.0 a	100	0.0 a	100
Control	90.0 b	–	89.6 b	–	89.4 b	–	88.5 b	–
60	0.0 a	100	0.0 a	100	0.0 a	100	0.0 a	100
Control	89.6 b	–	89.9 b	–	90.0 b	–	88.6 b	–
90	0.0 a	100	0.0 a	100	0.0 a	100	0.0 a	100
Control	89.6 b	–	90.0 b	–	89.8 b	–	88.9 b	–
120	0.0 a	100	0.0 a	100	0.0 a	100	0.0 a	100
Control	89.5 b	–	89.5 b	–	90.0 b	–	88.3 b	–

Values are means of three replications.
In a column, means followed by a common letter are not significantly different at 5 per cent ($P = 0.05$) level by DMRT.

periods of time (30, 60, 90 and 120 days); it proved to retain its antifungal effect from 30 days until the end of the storage period by exhibiting a 100% reduction in mycelial growth against all the tested pathogens. However, the control retained the maximum mycelial growth of 89.4 mm from the beginning to the end of the incubation period (Table 7).

4. Discussion

Plant products have assumed special significance in the present-day strategy of developing ecologically safe methods of plant disease management. Therefore, the present investigation was undertaken to test the efficacy of certain selected plant essential oils against *Curvularia lunata*, *Fusarium moniliforme*, *Bipolaris oryzae*, and *Sarocladium oryzae*, the seed-borne pathogens of rice in *in vitro* conditions.

The blotter method showed that, among the plant oils tested, *C. citratus* was found to be the best for rice seedlings. Seed soaking with *C. citratus* oil, thiram followed by *C. martinii* oil increased the seed germination percentage. Schwinn [31], who stated that any bio agents used as seed treatment purpose should protect both seed and soil-borne pathogens, control deep-seated seed-borne pathogens and stimulate germination and or enhance growth during the seedling stages.

The germination of rice seedlings raised from the seeds treated with all oils was better compared to that of seedlings from the untreated seeds. The enhancement of seed germination by the natural products might be due to several factors such as fungitoxic actions leading to killing of the pathogens present both internally and externally in the seeds. Similarly, Mishra and Dubey [32] reported that the non-phytotoxic nature of plant oil *C. citratus*, even at 1000 ppm, increases the germination of rice and of wheat seed. Ngufack et al. [28] reported that rice seeds treated with *C. citratus*, *T. vulgaris* and *O. gratissimum* recorded high germination with that of Dithane M-45 treated seeds. Similar kind of strong effect has previously been obtained with essential oil from *O. gratissimum* towards *F. moniliforme* in maize seeds reported by Tangne et al. [33]. Ravikumar [34] reported that rice seeds treated with palmarosa oil, carbendazim and followed by lemongrass oil increased the seed germination percentage. Seed treatment with *C. citrinus* oil significantly increased the emergence of the rice seedling by 16% [15,35]. This is to our knowledge the first report on *C. citratus*, *C. martinii* and *P. graveolens* oils as germination stimulants of rice seeds.

Rice seeds treated with three selected essential oils at a concentration of 0.1% gave the highest number of normal seedling and the lowest number of abnormal and fresh ungerminated seeds using the roll-towel method. Seed soaking with essential oil has the ability to migrate into the rice seed. Subsequently, it should protect against fungal infection and stimulate germination and enhance growth during the seedling stage [28].

This is a report available on the record of normal and abnormal seedlings in discoloured rice seeds after treatment with essential oils by the blotter method. But so far there was no report available for assessing the normal and abnormal seedlings by using plant oils as a seed treatment. This seems to be a new report.

These plant oils were formulated by using solvents, emulsifier Unitox® 60 and stabilizer. So far there was no report available for EC-formulated products of lemongrass, geranium, and palmarosa oils. Hence, this seems to be a new report. The present findings about new EC formulated plant oils are in agreement with the findings of many workers, are discussed below.

Sarala et al. [36] reported that palmarosa oil (0.1%) 80 EC and palmarosa + neem oil (1:2) 80 EC (0.1%) were more effective; these authors recorded the mycelial growth inhibition of 100 and 87.34% in *S. oryzae*, 100 and 92.40% in *D. oryzae* and 86.12% in *C. lunata*, respectively. Veerasamy (1997) developed EC formulations of ETCCA-60 EC (acetic acid) combined with *Eucalyptus terticornis* + *Trianthema portulacastrum* + citronella oil + $CaCl_2$, which were found to be effective against *Alternaria* leaf blight in brinjal. Narasimhan et al. [37] reported that neem oil 60 EC (Acetic acid) significantly inhibited the mycelial growth of *S. oryzae in vitro.* Rajappan et al. [38] developed neem-based EC formulations of NO 60 EC (acetic acid), formulations of neem oil, neem seed kernel extract, pungam oil and pungam cake, which can be used to control *Erysiphe polygoni*, causing green gram powdery mildew. Rajappan et al. [39] reported that neem oil 60 EC (citric acid), neem oil 60 EC (acetic acid) and neem oil + pungam oil 60 EC (citric acid) inhibited the mycelial

growth of *H. oryzae* and *P. oryzae.* Anusha [40] developed two EC formulations (60 EC) in *Lantana camera* at 2 and 4% that proved to be ineffective against *R. solani*, causing a sheath blight disease. Vanitha [24] reported that 30 and 40 EC formulations of winter green and lemongrass oils were significantly effective by recording 100% inhibition of mycelial growth and spore germination of *A. chlamydospora* causing the leaf blight of *Solanum nigrum*.

All the EC formulations (10, 20 and 30 EC) inhibited the mycelial growth of the test pathogens. Among these, 30 EC formulations of *C. citratus*, *C. martinii* and *P. graveolens* oil showed the maximum percentage of inhibition of the mycelial growth of *C. lunata*, *F. moniliforme*, *B. oryzae*, and *S. oryzae*. The other EC formulations were also effective although somewhat less in restricting the growth of test pathogens. The highest antifungal activity of EC formulation of *C. citratus*, *C. martinii* and *P. graveolens* could be related to the different modes of action of their major and known active compounds. Some plant oils have been reported to have high electrophilic properties that increase their reactivity with nucleophiles such as the protein sulfhydryl and amino groups of the pathogen [41]. The above results support the present findings.

The results of seed treatment with the 30 EC formulation of *C. citratus* oil revealed that it controlled the level of infection of *C. lunata*, *F. moniliforme*, *B. oryzae* and *S. oryzae* in rice seed by 66.0%, 60.4%, 66.0, 69.1%, respectively. The same trend was followed for rice variety White Ponni.

Similarly, Nguefack et al. [35] reported that rice seeds treated with '*C. citratus* and *O. gratissimum* significantly reduced the seed infection of *B. oryzae* in a range of 42 to 100%. Somda et al. [42] reported that *C. citratus* at a concentration of 6% was effective in controlling the seed-borne infection of *C. graminicola*, *P. sorghina*, and *F. moniliforme* in sorghum seed. Nguefack et al. [28] reported that rice seeds treated with essential oils of *O. gratissimum* and *T. vulgaris* controlled 100% seed infection by *B. oryzae* and that from *C. citratus* controlled 93% of the same infection. In another study, rice seeds treated with essential oils from *O. gratissimum*, *C. citratus* and *T. vulgaris* were equally effective as Dithane-M-45, in controlling the seed infection by 95–100%. Seed soaking with essential oil have the ability to migrate into the rice seed. Subsequently, it should protect against fungal infection.

These results indicated that the essential oils tested could potentially be control agents of the seed-borne fungi studied. The antifungal activity of these essential oils could be attributed to their contents in known antimicrobial compounds; such chemical compositions have been studied by Amram Zollo et al. [43]. Nguefack et al. [35], who found that *C. citratus* oil contained mostly citral a and b, by Tassou et al. [44] who studied the mode of action of essential oils and of their phenolics. They reported that terpenes containing alcoholic compounds are more effective against microorganism than the ones having an aldehydic function. The above results support the present findings.

Among the three EC formulations, seed soaking with (ADT 45 and White Ponni) 30 EC formulation of *C. citratus* oil showed the highest number of normal seedling, the lowest number of abnormal seedlings and fresh ungerminated seeds with the variety ADT 45. The same trend was observed for the rice variety White Ponni.

Seed soaking with essential oil have the ability to migrate into the rice seed. Subsequently, it should protect against from fungal infection and stimulate germination and enhance growth during the seedling stage. This is a report available on the record of normal and abnormal seedlings in discoloured rice seeds after treatment with essential oils by the blotter method. But, so far, there was no report available for assessing the normal and abnormal seedlings by using essential oils as a seed treatment. This seems to be a new report.

The results of seed soaking with 30 EC formulation of *C. citratus* oil revealed that showed increased seed germination, shoot length, root length and vigour of rice seedlings. Similarly, Ravikumar [34] reported that seed soaking with palmarosa oil, carbedazim followed by lemongrass oil increased seed germination, shoot length, root growth and vigour of rice seedlings. Nguefack et al. [28] reported that rice seeds treated with essential oils of *C. citratus*, *O. gratissimum*, and *T. vulgaris* increased the germination percentage and vigour of rice seedlings compare to untreated control.

Harish et al. [45] found that spraying rice plants twice with neem cake extract and *Nerium oleander* leaf extract in the field reduced the severity of brown spot by 70% and 53%, respectively, and increased the yield by 23% and 18%, respectively. Nguefack et al. [15] recorded that the combined use of the essential oil of *C. citratus* as a seed treatment and spraying the plants with 2% ethanol followed by 2% (w/v) aqueous extracts of *C. citrinus* or *C. citratus* increased the emergence, tillering, panicles/plant and the grain yield by 25–55% of the irrigated rice.

The vigour of rice seedlings raised from the seeds treated with *C. citratus* oil was better compared to that of seedlings from untreated seeds. The enhancement of seed germination and growth of seedlings by plant oils might be due to several factors such as fungitoxic actions leading to the killing of the pathogens present both internally and externally.

All three EC formulations of *C. citratus* oil reduced the seed-to-seedling transmission of *C. lunata*, *F. moniliforme*, *B. oryzae* and *S. oryzae*. Among them, 30 EC formulations were highly effective in reducing seed-to-seedling transmission of test pathogens. The same trend was followed for the rice variety White Ponni. Similarly, Nguefack et al. [28] reported that rice seeds treated with essential oils from *C. citratus*, *O. gratissimum* and *T. vulgaris* reduced the seed-to-seedling transmission of *H. oryzae* by 91%, 86% and 83%. In another treatment, the treatment of the seeds with essential oils from *C. citratus*, *O. gratissimum* and *T. vulgaris* was as effective in reducing the seed-to-seedling transmission of *F. moniliforme* as in reducing that of Dithane M-45. The transmission of *A. padwickii* from seed to seedling was reduced significantly by 76%, 79% and 85% with the essential oils from *C. citratus*, *T. vulgaris* and *O. gratissimum*, respectively, and 77% with Dithane M-45. Somda et al. [42] reported that the essential oil of lemongrass at 6% concentration was effective in reducing the seed-to-seedling transmission of *C. graminicola*, *P. sorghina*, and *F. moniliforme*. The above results support the present findings.

The 30 EC formulation of *C. citratus* oil showed 100% reduction in the mycelial growth of test pathogens even after 120 days of storage. This is in agreement with the findings of Narasimhan et al. [37], who reported that there was no significant difference between the efficacy of the freshly prepared and stored EC formulations of neem and pungam oil in arresting the mycelial growth of *S. orzae*; the efficacy was maintained even after 9 months of storage. Rajappan et al. [39] reported that neem oil 60 EC (acetic acid), neem oil 60 EC (citric acid) and neem oil + pungam oil 60 EC (citric acid) completely inhibited the mycelial growth of *H. oryzae* and *P. oryzae*, and were effective even after 9 months of storage. Thobunluepop [46] reported that rice seeds treated with clove oil (eugenol) plus chitosan–lingosulphonate polymer showed a strong inhibitory effect on seed-borne fungi (*Curvularia* sp., *F. moniliforme*, *B. oryzae*, *A. padwickii*, *A. flavus* and *A. niger*) and retained their antifungal effect even 5 months after storage. Thereafter, the inhibitory effect was reduced. Vanitha [24] reported that the EC formulation of wintergreen oil, lemongrass oil and their combination retained their antifungal effect even up to 60 days after storage with 100% reduction in the mycelial growth and spore germination of *A. chlamydospora*, causing leaf blight of *Solanum nigrum*. Further, the antifungal effect was reduced after 90 days of storage.

The effectiveness of these formulations pertains to materials based on *C. citratus* oil, but they contain additional materials that might be biologically active, like cyclohexanone, epichlorohydrin and Unitox 60®. The above findings are in agreement with the present investigation. These materials can prolong the shelf life of antifungal agents and maintain the effectiveness throughout the incubation period.

5. Conclusion

In modern agriculture, diseases are usually managed by fungicides. The ecological concern over the excessive use of fungicides and their high cost has motivated the farmers to select methods that are environmentally friendly and also relatively cheap. Hence, in the present investigation, essential oils were developed into formulation and screened against major rice seed-borne pathogens. Seed treatment with EC formulation of plant oils reduced the seed infection percentage and increased the germination percentage and vigour of rice seedlings. In present study, the *C. citratus* oil 30EC formulation showed encouraging results in terms of its antifungal activity against major seed-borne pathogens of rice and of inhibition of the seed-to-seedling transmission of the fungus; in addition it increased the seed germination percentage also. Hence, the *C. citratus* oil formulation can serve as a natural fungicide or as a template for the synthesis of novel fungicides; however future studies are required to ensure their efficacy at the field level.

Acknowledgement

The authors are highly grateful to the Tamil Nadu State Council for Science and Technology, Chennai, for providing the grant and opportunity to carry out this work.

References

[1] FAO 2013. faostat.fao.org/site/339/default.aspx.
[2] A. Anandan, A. Mahender, R. Kumari, S.K. Pradhan, H.N. Subudhi, S. Thirugnanakumar, O.N. Singh, Appraisal of genetic diversity and population structure in assorted rice genotypes for early seedling vigour trait linked markers, Oryza 53 (2016) 113–125.
[3] IRRI, International Rice Research Institute: IRRI world Rice Statistics (WRS), Facts and Figures, 2008, 1960–2010.
[4] G.A. Fakir, M.A.T. Mia, The quality of farmer-saved rice seeds in Bangladesh, in: National Workshop on Seed Health Improvement, BARC, Dhaka, 7 July, 2004.
[5] N. Ora, N.A.N. Faruq, M.T. Islam, N. Akhtar, M.M. Rahman, Detection and identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh, Middle-East J. Sci. Res. 10 (2011) 482–488.
[6] IRRI, Standard Evaluation System of Rice, International Rice Testing Programme Los Banos, Phillipines, 1987.
[7] G.A. Fakir, An Annotated List of Seed-Borne Disease in Bangladesh, Seed Pathology Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, 2000.
[8] D.R. Fravel, K.L. Deahl, J.R. Stommel, Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides, Biol. Control 34 (2005) 165–169.
[9] B. Cebolla, J. Busto, A. Ferrer, A. Miguel, J.V. Maroto, Methyl bromide alternatives on horticultural crops, Acta Hortic. 532 (2000) 237–242.
[10] W.T. Song, L.G. Zhou, C.Z. Yang, X.D. Cao, L.Q. Zhang, X.L. Liu, Tomato Fusarium wilt and its chemical control strategies in a hydroponic system, Crop Prot. 23 (2000) 243–247.
[11] A. Sokmen, B.M. Jones, M. Erturk, The in vitro antibacterial activity of Turkish medicinal plants, J. Ethnopharmacol. 67 (1999) 79–86.
[12] A. Muthukumar, R. Udhayakumar, R. Naveenkumar, Eco friendly management of damping-off of solanaceous crops caused by Pythium species, in: P. Kumar, et al. (Eds.), Current Trends in Plant Disease Diagnostics and Management Practices, Fungal Biology, Springer International Publishing, Switzerland, 2016 , pp. 49–90. , http://dx.doi.org/10.1007/978-3-319-27312-9_3.
[13] M. Hashem, A.M. Moharama, A.A. Zaie, F.E.M. Saleh, Efficacy of essential oils in the control of cumin root rot disease caused by *Fusarium* spp, Crop Prot. 29 (2010) 1111–1117.
[14] A. Muthukumar, K. Sanjeekumar, Chemical composition and antifungal activity of essential oils against die-back of hippeastrum, Indian J. Hortic. 69 (2012) 385–389.
[15] G.J. Nguefack, G.E. Wulff, J.B.L. Dongmo, F.R. Fouelefack, F. Daniel, M.B.O. Joseph, J. Torp, Effect of plant extracts and an essential oil on the control brown spot disease, tillering, number of panicles and yield increase in rice, Eur. J. Plant Pathol. 137 (2013) 871–882.
[16] Y. Oka, S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv, Y. Spiegel, Nematicidal activity of essential oils and their components against the root-knot nematode, Phytopathology 90 (2000) 710–715.
[17] J. Nguefack, I. Somda, C.N. Mortensen, P.H. Amvam Zollo, Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (*Oryza sativa* L.), Seed Sci. Technol. 33 (2005) 397–407.
[18] H. Alilou, M. Akssira, L.I. Hassani, B. Chebli, A. El Hakmoui, Chemical composition and antifungal activity of *Bubonium imbricatum* volatile oil, Phytopathol. Mediterr. 47 (2008) 3–10.
[19] R. Naveenkumar, A. Muthukumar, G. Sangeetha, K.V. Neha, Antifungal activity of plant oils against major seed-borne fungi of rice, Oryza 53 (2016) 174–180.
[20] S. Gangopadhyay, K.S. Kapoor, Control of *Fusarium* wilt of okra with seed treatment, Indian J. Mycol. Plant Pathol. 7 (1977) 147–149.
[21] Y.L. Nene, P.N. Thapliyal, Fungicides in Plant Diseases Control, Oxford and IPH. Publishing Co. Pvt. Ltd., New Delhi, 1993.
[22] ISTA, International Seed Testing Association, ISTA Handbook on Seedling Evaluation, 3rd ed., 2003.
[23] O.F.A. Ibiam, C.I. Umechuruba, A.E. Arinze, A survey of seed borne fungi associated with seeds of rice (*Oryzae sativa* L. FARO 12, 15 and 29) in storage and the field in Afikpo North local Government area of Ebonyi State, Sci. Afr. 7 (2008) 1–4.
[24] S. Vanitha, Developing new botanical formulation using plant oils and testing their physical stability and antifungal activity against *Alternaria chlamydospora* causing leaf blight in *Solanum nigrum*, Res. J. Agric. Sci. 1 (2010) 385–390.
[25] R. Meenakshisundaram, Development of Neem Oil Formulations, M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 1991, pp. 19–26.
[26] H.L. Burnett, B.B. Hunter, Illustrated Genera of Imperfect Fungi, 4th ed., Burgess Publishing Co., Minneapolis, 1991, p. 241.

[27] A.A. Baki, J.D. Anderson, Physiological and biochemical deterioration of seeds, in: T. Kozlowski (Ed.), Seed Biology, vol. 2, Academic Press, New York, 1972, pp. 203–215.
[28] J. Nguefack, V. Leth, J.B. Lekagne, J. Dongmo, J. Torp, P.H. Amvam Zollo, S. Nyasse, Use of three essential oils as seed treatments against seed borne fungi of rice (*Orzae sativa* L.) American-Eurasian, J. Agric. Environ. Sci. 4 (2008) 5–554.
[29] S. Veerasamy, Studies on Management of Leaf Blight Disease of Brinjal (*Solanum melongena*) Caused by *Alternaria solani* (EII. And Mont.) Jones and Grout, Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 1997, p. 126.
[30] K.A. Gomez, A.A. Gomez, Statistical Procedure for Agricultural Research, John Wiley and Sons, New York, NY, USA, 1984.
[31] F.J. Schwinn, Seed treatment – a panacea for plant protection, in: Seed treatment Trevor (Ed.), Proceedings of a Symposium of the British Crop Protection Council and Pesticides Group of the Society of Chemical Industry, Canterbury, (1994), pp. 3–13.
[32] A.K. Mishra, N.K. Dubey, Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities, App. Environ. Microbiol. 60 (1994) 1101–1105.
[33] A. Tangne, J. Nguefack, P.H. Amvam Zollo, Natural control of fungi and mycotoxin in grains-means of reducing human and animal contamination, J. Appl. Sci. South. Afr. 6 (2000) 37–44.
[34] S. Ravikumar, Eco Friendly Management of Rice Sheath Blight Disease With Plant Essential Oils, M. Sc., (Ag.) Thesis, Annamalai University, Chidambaram, Tamil Nadu, India, 2012, p. 103.
[35] J. Nguefack, S.K. Nguikwie, D. Fotio, Fungicidal potential of essential oils and fractions from *Cymbopogon citratus*, *Ocimum gratissimmum* and *Thymus vulgaris* to control *Alternaria padwickii* and *Bipolaris oryzae*, two seed-borne fungi of rice (*Oryza sativa* L.), J. Essential Oil Res. 17 (2007) 581–587.
[36] L. Sarala, M. Muthusamy, K. Karunanithi, M. Karthikeyan, Efficacy of plant oils against the pathogens of grain discolouration of rice, Crop Res. 23 (2002) 21–23.
[37] V. Narasimhan, K. Rajappan, C. Usha Malini, A. Abudul Kareem, Efficacy of EC formulation of neem oil and pungam oil for the management of sheath rot disease of rice, Phytoparasitica 26 (1998) 301–306.
[38] K. Rajappan, V. Mariappan, A. Abdul Kareem, Development and evaluation of EC and dust formulation of plant derivatives against powdery mildew, Annu. Plant Prot. Sci. 7 (1999) 91–119.
[39] K. Rajappan, C. Usha Malini, N. Subramanian, V. Narasimhan, A. Abudul Kareem, Management of grain discoloration of rice with solvent free EC formulations of neem and pungam oils, Phytoparasitica 29 (2001) 171–174.
[40] B. Anusha, Development of Botanical Fungicide(s) for the Management of Rice Sheath Blight, M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 2003, pp. 110–117.
[41] F. Neri, M. Mari, S. Brigati, Control of *Penicillium expansum* by plant volatile compounds, Plant Pathol. 55 (2006) 100–105.
[42] I. Somda, V. Leth, P. Sereme, Antifungal effect of *Cymbopogon citratus*, *Eucalyptus camaldulensis* and *Azadirachta indica* oil extracts on sorghum seed borne fungi, Asian J. Plant Sci. 6 (2007) 1182–1189.
[43] P.H. Amram Zollo, L. Biyiti, F. Tchoumbougnang, C. Menut, G. Lamaty, P.H. Bouchet, Aromatic plants of tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon, Flavour Fragr. J. 13 (1998) 107–114.
[44] C. Tassou, K. Koutsoumanis, G.J.E. Nychas, Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil, Food Res. Int. 33 (2000) 273–280.
[45] S. Harish, D. Saravanakumar, R. Radjacommare, E.G. Ebenezer, K. Seetharaman, Use of plant extracts and biocontrol agents for the management of brown spot disease in rice, Biocontrol 53 (2008) 555–567.
[46] P. Thobunluepop, The inhibitory effect of the various seed coating substances against rice seed borne fungi and their shelf life during storage, Pak. J. Biol. Sci. 12 (2009) 1102–1110.