PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp4): 01–11
https://doi.org/10.14719/pst.5395

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RESEARCH ARTICLE

Eco-friendly approaches for controlling *Echinochloa colona* in rice ecosystems using natural herbicides

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OPEN ACCESS

ARTICLE HISTORY
Received: 30 September 2024
Accepted: 22 October 2024
Available online
Version 1.0 : 29 November 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE
Salman MM, Gurusamy A, Subramanian E, Prabhaharan J, Arunachalam P, Radha P. Eco-friendly approaches to controlling *Echinochloa colona* in rice ecosystems using natural herbicides. Plant Science Today.2024;11(sp4):01-11. https:/doi.org/10.14719/pst.5395

Abstract

Echinochloa colona, a highly invasive weed, poses significant challenges to rice cultivation globally and is usually managed effectively through chemical herbicides. Conventional chemical herbicides have contributed to environmental degradation and increased herbicide resistance. This study explores the potential of natural herbicides derived from plant extracts as eco-friendly alternatives for sustainable weed management. Ten plant samples, including *Calotropis gigantea, Ocimum tenuiflorum* and *Prosopis juliflora*, were collected and their allelochemicals were extracted using Soxhlet extractor with methanol and hexane solvents. The efficacy of these extracts in inhibiting *E. colona* germination was tested through pot culture and laboratory assays. GC-MS analysis identified key allelochemicals responsible for the phytotoxic effects, leading to the formulation of natural herbicides in the form of Emulsifiable Concentrates (EC). Field experiments were conducted to assess the performance of these formulations in rice ecosystems. Results indicated that pre-emergence application of 50% EC formulation of 10% methanolic extract of *Ocimum tenuiflorum* + one hand weeding at 25 DAS showed significant reduction in weed density, dry weight, weed index and increase in weed control efficiency, crop yield and highest benefit cost ratio though further optimization is required to improve formulation stability. This study demonstrates the potential of natural herbicides as effective and sustainable tools for managing *E. colona* in rice fields, providing an alternative to synthetic chemicals. The findings underscore the need for continued research into the development of durable and efficient natural herbicide formulations.

Keywords

weed; allelochemicals; natural herbicides; sustainable; formulations

Introduction

Echinochloa species are notorious weeds in many economically important crops worldwide. Among them, *Echinochloa colona* (L.) is particularly aggressive and has been affecting crops and vegetables across over 60 countries especially in rice fields (1). This weed serves as an alternative host for diseases, insects and nematodes. *E. colona* primarily reproduces through seeds with a shorter dormancy period and begins emerging 2-3 DAS, reaching the two-leaf stage by 8 DAS (2). It also reproduces vegetatively

through its nodes (3). This weed is prevalent in dry-seeded, wet-seeded and transplanted rice, causing yield losses of 27% to 62% (4) and depleting 60% to 80% of soil nitrogen (5). Due to its resemblance to rice during the seedling stage, it is often called "jungle rice". The critical period of interference in rice occurs between 20 to 40 DAS, making early weed management crucial to avoid economic losses (6).

Weed control is crucial for rice cultivation and usually requires a combination of chemical and manual techniques to manage it effectively. However, the morphological similarity of *E. colona* with rice renders its manual weeding laborious and expensive (7). On the other hand, high reliance on synthetic herbicides has resulted in environmental pollution and established a high level of resistance in *Echinochloa* spp against herbicides. In this regards, modern agriculture advocates for methods such as utilizing natural herbicides derived from plant extracts to promote environment friendly weed management practices. These weed killing substances are both efficient and environmentally friendly as they prevent the growth of weeds by using a process called allelopathy, where plants release chemicals that impact other plant species growth and germination cycles positively or negatively. The use of this method shows significant potential in creating novel herbicides, for managing *E. colona* in rice ecosystem. To address *E. colona* management in an eco-friendly manner, this study standardizes extraction procedures of allelochemical using a Soxhlet extractor, identifies allelochemicals through GC-MS analysis, formulates natural herbicides as emulsifiable concentrates (EC) and assesses their efficacy in lab and field studies.

Materials and Methods

Plant sample

The plant samples listed in Table 1 were collected from areas in and around the Agricultural College and Research Institute, Madurai, Tamil Nadu, India. The institute is situated in the southern agroclimatic zone of Tamil Nadu, at coordinates 9°54' N latitude and 78°54' E longitude, with an elevation of 147 meters above mean sea level (MSL). The soil type in this region is sandy clay loam. Over the past 30 years, the average weather conditions at the site have recorded an annual rainfall of 846 mm distributed over 43 rainy days, with average maximum and minimum temperatures of 33.7°C and 23.8°C, respectively. The area also experiences a mean daily pan evaporation of 6.2 mm and relative humidity levels averaging 80% at 07:14 hours and 60% at 14:14 hours. Various parts such as leaves, stems, roots, flowers and fruits were gathered to ensure comprehensive phytochemical representation. Samples were washed, dried in a well-ventilated, shaded area for 30-40 days until the samples became brittle and then powdered for further use (8).

Allelochemical extraction procedure

Soxhlet extraction process was employed to extract allelochemicals. Thirty grams of dried, powdered plant material was placed in a filter paper thimble within the Soxhlet apparatus. Methanol and hexane (HPLC grade) were used as solvents. Extraction continued for 8-12 hours until the solvent appeared clear, ensuring efficient extraction of allelochemicals (9). Post-extraction, solvents were evaporated under reduced pressure at 40°C using a rotary evaporator and extracts were stored at -20°C until analysis (10).

Germination assay in Pot culture experiment

E. colona seeds were collected, cleaned, and air-dried. For pot experiments, a soil-sand-compost mix (1:1:1) was prepared and 200g of the mix was added to each pot. Ten seeds were sown per pot at a 2 cm depth. Methanolic and hexane extracts were applied at 5% concentration, while control pots received water only. Pots were arranged in a completely randomized design (CRD) with two replications. Germination percentage was calculated using (11)

$$\text{Germination Percentage} = (\text{Number of Seeds Germinated} / \text{Total Number of Seeds Sown}) \times 100 \quad \text{(Eqn.1)}$$

Germination assay in laboratory studies

For laboratory studies, germination sheets were placed in Petri dishes, with ten healthy *E. colona* seeds per dish. Seeds were treated with 5% concentrations (0.5 ml of extracts dissolved in 10 ml of water) of methanolic and hexane plant extracts. Control dishes received water only. After 7 days, germination percentage and radicle length were assessed by counting the number of sprouted seeds and measuring radicle lengths and listed in Table 2. Both pot culture and laboratory experiments showed lower germination percentage. The root and shoot length of E. *colona* seeds were observed in T_9 (methanolic extracts of Holy basil), followed by T_8 (methanolic extracts of Milkweed) and T_5 (methanolic extracts of Mesquite). Allelochemicals responsible for this inhibition were identified through GC-MS analysis of methanolic extracts of these plants. Subsequently, new natural herbicides were formulated by using these extracts in the form of emulsifiable concentrates (EC).

Table 1. List of selected plants and their plant parts for allelochemical extraction

Sl. No	Plants samples	Botanical name	Plant parts collected
1	Sorghum	*Sorghum bicolor*	Leaves, stem, inflorescences,
2	Sunflower	*Helianthus annuus*	Whole plant
3	Thumba	*Leucas aspera*	Whole plant
4	Goat weed	*Ageratum conyzoides*	Whole plant
5	Holy basil	*Ocimum tenuiflorum*	Whole plant
6	Thorn-apple	*Dautura metal*	Whole plant
7	Chinese chaste tree	*Vitex negundo*	Leaves, stem, inflorescence,
8	Milkweed	*Calotropis gigantea*	Leaves, stem, inflorescence, flower and fruit
9	Mesquite	*Prosopis juliflora*	Leaves, bark, inflorescence, flower and fruit
10	Gum trees	*Eucalyptus globulus*	Leaves, bark, inflorescence, flower and fruit

Table 2. Effect of methanolic and hexane extracts of selected plants in the germination of *Echinochloa colona* seeds in both Pot culture and laboratory experiment

Treatments	Germination (percentage) in Pot culture experiment	Germination (percentage) in laboratory experiment	Root length in cm	Shoot length in cm
T_1 - Methanolic extracts of Sorghum	80	60	5.29	7.40
T_2- Methanolic extracts of Sunflower	70	70	5.36	7.51
T_3 - Methanolic extracts of Thumba	70	50	5.24	7.48
T_4- Methanolic extracts of Goat weed	80	60	5.38	7..43
T_5- Methanolic extracts of Mesquite	30	20	4.13	4.89
T_6- Methanolic extracts of Thorn-apple	90	80	5.64	7.66
T_7- Methanolic extracts of Chinese chaste tree	70	70	5.57	7.60
T_8- Methanolic extracts of Milkweed	20	30	3.9	4.78
T_9- Methanolic extracts of Holy basil	10	10	3.5	4.56
T_{10}- Methanolic extracts of gum trees	60	70	5.5	7.56
T_{11}- Hexane extracts of Sorghum	80	90	5.7	7.83
T_{12} - Hexane extracts of Sunflower	90	80	5.65	7.72
T_{13}- Hexane extracts of Thumbai	90	90	5.99	7.78
T_{14} - Hexane extracts of Goat weed	90	90	5.78	7.62
T_{15}- Hexane extracts of Mesquite	40	60	4.6	4.97
T_{16}- Hexane extracts of Thorn-apple	90	100	6.0	7.90
T_{17}- Hexane extracts of Chinese chaste tree	80	80	5.85	7.88
T_{18}- Hexane extracts of Milkweed	30	20	4.8	4.95
T_{19} - Hexane extracts of Holy basil	50	40	4.5	4.88
T_{20}- Hexane extracts of gum tree	60	60	5.8	7.54
T_{21}- Control	90	100	7.34	9.06

Formulation procedure

To formulate a 50% Emulsifiable Concentrate (EC), equal volumes of methanolic plant extracts were mixed with distilled water to achieve a 50% extract concentration. The mixture was stirred at 750 rpm for 15 minutes using a magnetic stirrer. A 30 ml of cyclohexane was slowly added to form a coarse emulsion, with continuous stirring at 750 rpm for 30 minutes (12). After this, 30 ml each of Tween 80 and Triton X-100 surfactants were added and stirred at 450 rpm for 10 minutes. The mixture was transferred to an ultrasonicator and ultrasonically emulsified for 30 minutes at 25 kHz (13). Finally, the emulsion was homogenized at 6000 rpm for 30 minutes using a high shear homogenizer to achieve a stable and finely refined EC, ready for field efficacy studies.

Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis was conducted using a Shimadzu GC-MS-QP2020 NX system with an SH-5MS column available at Madurai Kamarajar University. The analyses were performed in split injection mode with a flow rate of 1.50 mL/min under temperature of 230°C (14) and library matching was done using NIST 2022 software for precise identification of allelochemicals.

Field experiment

Field experiments on managing *E. colona* with natural herbicides in the rice ecosystem were conducted at Agricultural College and Research Institute, Madurai, Tamil Nadu, India during the summer of 2024. Throughout the study, the site received 425.4 mm of rainfall distributed over seventeen rainy days. The mean evaporation rate was recorded at 5.6 mm. Climatic conditions featured mean maximum and minimum temperatures of 35.4°C and 23.6° C, respectively. Relative humidity (RH) ranged from 85.7% in the morning to 55.8% in the afternoon. The area experienced an average of 7.8 hours of bright sunshine daily, accompanied by an average wind speed of 5.3 km/ hr. The newly formulated natural herbicides are sprayed as pre-emergence (PE) at three different concentration 5, 7.5, 10% followed by one hand weeding at 25 DAS. The experiment was conducted using a randomized block design (RBD) with three replications. Treatments as follows: **T_1-** PE application of 5% Methanolic extract (50% EC) of *Calotropis gigantea*+ Hand weeding at 25 DAS, **T_2-** PE application of 7.5% Methanolic extract (50% EC) of *Calotropis gigantea*+ Hand weeding at 25 DAS, **T_3-** PE application of 10% Methanolic extract (50% EC) of *Calotropis gigantea*+ Hand weeding at 25 DAS , **T_4-** PE application of 5% Methanolic extract (50% EC) of *Prosopis juliflora* + Hand weeding at 25 DAS, **T_5-** PE application of 7.5% Methanolic extract (50% EC) of *Prosopis juliflora* + Hand weeding at 25 DAS, **T_6-** PE application of 10% Methanolic extract (50% EC) of *Prosopis juliflora* + Hand weeding at 25 DAS, **T_7-** PE application of 5% Methanolic extract (50% EC) of *Ocimum tenuiflorum* + Hand weeding at 25 DAS, **T_8-** PE application of 7.5% Methanolic extract (50% EC) of *Ocimum tenuiflorum* + Hand weeding at 25 DAS, **T_9-** PE application of 10% Methanolic extract (50% EC) of *Ocimum tenuiflorum* + Hand weeding at 25 DAS, **T_{10}-** Weed free check , **T_{11}-** Weedy check

Observation

Parameters such as weed density, weed dry weight, weed control efficiency are collected at regular intervals at 15, 30 and 45 DAS. The weed density was observed by using a quadrant at randomly in each plot and weeds from four 1 m^2 quadrants per plot were collected, oven-dried at 70°C until a constant weight was reached. The final dry weight of the weeds was recorded and expressed in g m^{-2}.

Weed control efficiency

Weed control efficiency (WCE) was calculated by using the below formulae: (15)

$$WCE\ (\%) = \frac{\text{Dry weight of weeds in control plots - Dry weight of weeds in treated plots}}{\text{Dry weight of weeds in control plots}} \times 100 \quad \text{(Eqn.2)}$$

Weed Index (WI)

The Weed Index (WI) was determined using the formula established by (16).

$$\mathbf{WI = (X - Y / X) \times 100} \quad \text{.....} \quad \text{(Eqn.3)}$$

Where, X= Yield from weed-free plot, Y = Yield from treated plot

Economics

Net return, Benefit cost ratio and Cost of cultivation were calculated with the help of the current market price of the inputs and produce (17)

$$\text{Benefit cost ratio (BCR)} = \frac{\text{Gross return (Rs/ha)}}{\text{Cost of cultivation (Rs/ha)}} \quad \text{(Eqn.5)}$$

$$\text{Net return (Rs/ha)} = \text{Gross return (Rs/ha) - cost of cultivation (Rs/ha)} \quad \text{(Eqn.4)}$$

Statistical analysis

The observations of various characteristics were analyzed statistically according to the methodology outlined by (18). The data were organized and presented in tables, then subjected to statistical evaluation. Treatment effects were assessed using two-way ANOVA (Analysis of covariance) with AGRES software and significance was determined at a critical difference (CD) with a probability level of p = 0.05.

Results

Weed flora

In the rice ecosystem, grasses such as *E. colona* was observed to be more dominant among the weed species compared to sedges and broad-leaved weed. In addition to *E. colona* other weed species such as *Cyperus difformis, Cyperus rotundus, Ludwigia parviflora and Panicum repens* were predominantly observed in the field.

Gas Chromatography-Mass Spectrometry findings

A total of 32 chemical compounds were found in the methanolic extracts of *Calotropis gigantea* by GC-MS analysis (Table 3, Fig 1), some of which have allelopathic and phytotoxic properties that may inhibit the germination and growth of *E. colona*. Some fatty acids were found to include Oleic Acid, 9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester, n-Hexadecanoic Acid, Hexadecanoic Acid and 8,11,14-Docosatrienoic Acid. Furthermore, two phenolic compounds and terpenoids, namely Methyl eugenol, Phytol and 4H-Pyran -4-one, 2, 3-dihydro-3,5-dihydroxy and Neophytadiene, were identified as potentially significant factors influencing the germination and growth of *E. colona* seeds Table 3.1.

GC-MS analysis of methanolic extracts from *Prosopis juliflora* revealed the presence of 35 chemical compounds (Table 4, Fig. 2). A few of them have been identified as allelochemicals, including Octadecanoic Acid, 4-Vinylphenol, 2-Methoxy-4-vinylphenol, Neophytadiene, Stigmasterol, Campesterol, Diethyl Phthalate and Alpha-Methyl Mannofuranoside (Table 4.1).

GC-MS analysis of *O. tenuiflorum* had identified nearly 40 bioactive compounds (Table 5, Fig. 3) including allelochemicals such as methyl eugenol, caryophyllene, humulene and stigmasterol in Table 5.1. *O. tenuiflorum* extracts is a possible candidate for the development of natural herbicides, especially for the control of *E. colona.*

Weed density and weed dry weight

The lowest *E. colona* weed density and dry weight was consistently observed in the weed-free check (T10) as presented in Table 6 & 7, across densities of 1.44, 1.23 and 1.52 weeds m^{-2} and dry weights of 1.13, 1.13 and 1.37 g m^{-2}at 15, 30 and 45 DAS respectively, exhibited significant weed suppression. A major inspired cutoff amongst treatments, was T_9 (10% methanolic extract [50% EC] *O. tenuiflorum* + hand weeding at 25 DAS) with the recorded densities of 3.4, 2.53 and 38.43 weeds m^{-2} and dry weights of 1.55, 1.57 and 5.42 g m^{-2}, respectively at 15, 30 and 45 DAS. It was characteristic of the phytotoxicity of *O. tenuiflorum* to *E. colona* germination, root and shoot growth was able to reduce in both weed density and dry weight. However, the growth of weed at 45 DAS hints to declining in efficacy of natural herbicides, suggesting the need for additional management measures. The weedy check (T_{11}) had the highest relative density and dry weight across measurements, with density of 5.33, 5.26 and 7.20 weeds m^{-2} and dry weight of 5.26, 5.26 and 7.20 g m^{-2} at 15, 30 and 45 DAS respectively and with substantial weed densities with no controls. Other treatments T_4 (5% methanolic extract [50% EC] of *Prosopis juliflora* handweeding at 25 DAS), showed higher weed density and dry weight even with less concentrated *P. juliflora* than T_9 and the weed-free check. These findings coincide with previous research on the herbicidal activity of plant extracts of *O. tenuiflorum* on controlling *E. Colona.*

Weed Control Efficiency

From Table 8, the weed-free control T_{10} showed the maximum control efficiency with almost complete suppression of *E. colona*. In addition, T_9 (10% Methanolic extract (50% EC) of *O. tenuiflorum* + hand weeding at 25 DAS) proved to be the most potent treatment out of the botanical treatments and recorded the control efficiency of 93.18, 92.74 and 44.00% at 15, 30 and 45 DAS respectively. This indicates a high inhibitory potential of *O. tenuiflorum* against *E. colona*. On the contrary, the control efficacy recorded in weedy check (T_{11}) was 0 percent, highlighting the need for effective weed management. Lower levels of concentration of *Calotropis gigantea* (T_1, T_2) and *P. juliflora* (T_4, T_5) recorded relatively poor effectivity against *E. colona* in comparison to *O. tenuiflorum*. A reduction in control efficiency at 45 DAS for all botanicals most treatments could indicate either regrowth of *E. colona*, or a decrease in efficacy over time.

Table 3. List of bioactive compounds present in GC-MS analysis of methanolic extracts of *Calotropis gigantea*

Peak	R. Time	Area	Area percent	Height percent	Name of compounds
1	4.907	143018	0.64	0.45	Nanofin
2	4.999	55135	0.25	0.30	3-Ethyl-1,3-dimethyldiaziridine (cis)
3	5.544	180407	0.81	0.30	Diglycerol
4	6.395	64846	0.29	0.33	1-Butanamine, 2-methyl-N-(2-methylbutylid
5	6.548	161888	0.73	0.62	Piperidine, 2,3-dimethyl-
6	8.056	105726	0.48	0.51	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-
7	8.555	144497	0.65	0.60	5-Methoxypyrrolidin-2-one
8	9.492	233428	1.05	0.46	Benzeneacetic acid, hexyl ester
9	9.965	199189	0.90	0.78	9-Oxabicyclo [3.3.1] nonan-2-one, 5-hydroxy
10	10.496	166860	0.75	0.62	Phenethyl piperidino sulfone
11	11.316	211382	0.95	0.86	DL-Proline, 5-oxo-, methyl ester
12	11.635	89382	0.40	0.49	Methyleugenol
13	12.035	591947	2.66	1.73	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
14	12.996	1074131	4.83	1.90	DL-Proline, 5-oxo-
15	13.895	64965	0.29	0.32	1,2,3,4-Tetrahydro-cyclopenta[b]indole
16	13.986	376224	1.69	1.60	Diethyl Phthalate
17	15.879	88885	0.40	0.43	Tetradecanoic acid
18	16.740	262434	1.18	1.06	Neophytadiene
19	17.005	180812	0.81	0.39	11-Hydroxy-11-methyl-tricyclo [4.3.1.1(2,5)]
20	17.625	213441	0.96	1.18	Hexadecanoic acid, methyl ester
21	17.762	109142	0.49	0.58	9-Hexadecenoic acid
22	17.963	4606098	20.73	22.73	n-Hexadecanoic acid
23	18.922	64713	0.29	0.31	Heptadecanoic acid
24	19.248	318240	1.43	1.90	9,12-Octadecadienoic acid (Z, Z)-, methyl est
25	19.307	660357	2.97	3.46	8,11,14-Docosatrienoic acid, methyl ester
26	19.411	661786	2.98	3.37	Phytol
27	19.595	2171809	9.77	14.24	10E,12Z-Octadecadienoic acid
28	19.648	7367188	33.16	31.28	Oleic Acid
29	19.856	1222027	5.50	5.08	Octadecanoic acid
30	21.601	78518	0.35	0.38	Eicosanoic acid
31	24.235	53827	0.24	0.28	Batilol
32	25.203	296534	1.33	1.47	Squalene
		22218836	100.00	100.00	

Table 3.1: List of potential allelochemicals from methanolic extracts of *Calotropis gigantea*

Potential allelochemicals	Molecular formula	Reference
n-Hexadecanoic Acid (Palmitic Acid)	$C_{16}H_{32}O_2$	
Hexadecanoic Acid Methyl Ester (Methyl Palmitate)	$C_{17}H_{34}O_2$	(24)
Oleic Acid	$C_{18}H_34O_2$	
9,12-Octadecadienoic Acid (Z, Z)-, Methyl Ester	$C_{19}H_{34}O_2$	
8,11,14-Docosatrienoic Acid, Methyl Ester	$C_{23}H_{40}O_2$	
Methyl eugenol	$C_{11}H_{14}O_2$	(29)
Phytol	$C_{20}H_{40}O$	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy	$C_5H_6O_3$	
Neophytadiene	$C_{20}H_{38}$	

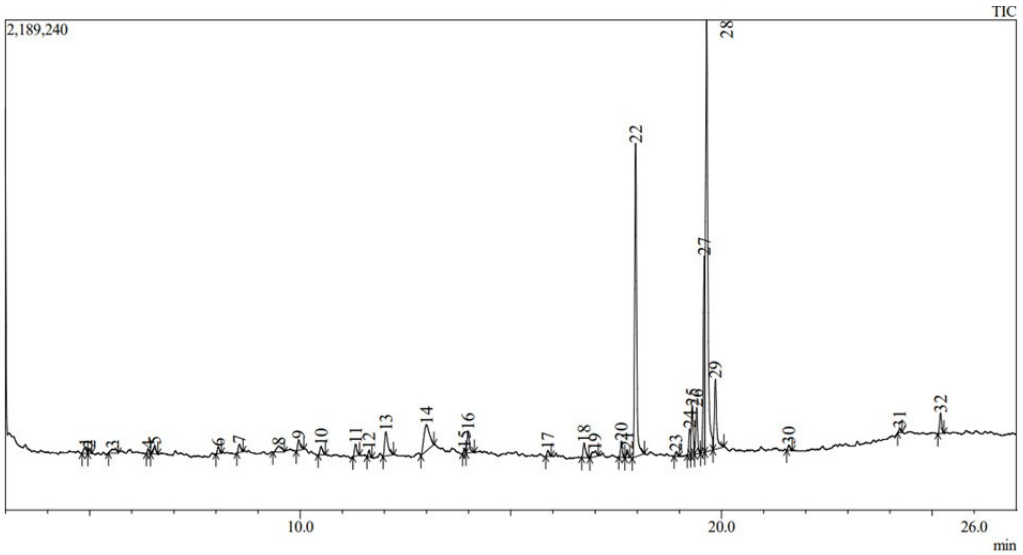

Fig. 1. The GC-MS chromatogram of methanolic extracts from *Calotropis gigantea*

Table 4. List of bioactive compounds present in GC-MS analysis of methanolic extracts of *Prosopis juliflora*

Peak	R. Time	Area	Area percent	Height percent	Name of compounds
1	7.025	275561	0.08	0.25	1-Octene, 4-methyl-
2	8.052	396949	0.12	0.46	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-
3	9.132	741995	0.22	0.82	4-Vinylphenol
4	10.485	534038	0.16	0.45	2-Methoxy-4-vinylphenol
5	13.179	421394	0.12	0.27	1[5'-(Hydroxymethyl)furfuryl] pyrrolidine
6	13.352	746478	0.22	0.43	3-Buten-2-ol, 2-methyl-4-(1,3,3-trimethyl-7-
7	13.661	401322	0.12	0.40	Phenol, 4-ethenyl-2,6-dimethoxy-
8	13.983	299132	0.09	0.34	Diethyl Phthalate
9	15.900	151242405	44.14	16.50	. alpha. -Methyl mannofuranoside
10	15.965	67007076	19.56	16.38	. alpha. -Methyl mannofuranoside
11	16.567	59497003	17.36	15.09	2-O-Methyl-D-mannopyranosa
12	16.740	4455072	1.30	2.23	Neophytadiene
13	17.000	813141	0.24	0.50	9-Octadecyne
14	17.184	306973	0.09	0.33	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	17.630	349423	0.10	0.46	Hexadecanoic acid, methyl ester
16	17.975	10010677	2.92	9.66	n-Hexadecanoic acid
17	18.272	896598	0.26	0.84	trans-Sinapyl alcohol
18	19.305	1447090	0.42	1.12	9,12,15-Octadecatrienoic acid, methyl ester,
19	19.417	4224994	1.23	4.65	Phytol
20	19.661	19145986	5.59	15.16	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
21	19.864	4728351	1.38	3.54	Octadecanoic acid
22	20.941	345436	0.10	0.38	Dimethylaminoethyl palmitate
23	21.606	345102	0.10	0.29	Eicosanoic acid
24	22.478	2039316	0.60	1.30	2-Dodecanone, 12-(5-hydroxy-6-methyl-2-pi
25	22.813	215155	0.06	0.26	Hexadecanoic acid, 2-hydroxy-1-(hydroxym)
26	22.927	2381757	0.70	2.33	2-Dodecanone, 12-(5-hydroxy-6-methyl-2-pi
27	23.105	239155	0.07	0.25	2-Tetradecanone, 14- [5-hydroxy-1,6-dimeth
28	24.240	2132681	0.62	0.98	7-Hexadecenal, (Z)-
29	24.400	826159	0.24	0.59	4,5,7,8-Tetrahydro-1,5-diazocine-2,6(1H,3H)
30	24.508	1959353	0.57	0.76	Campesterol
31	24.850	646346	0.19	0.24	Octadecanoic acid, octyl ester
32	25.074	977812	0.29	0.61	Stigmasterol
33	25.205	1048724	0.31	1.12	Squalene
34	26.051	548981	0.16	0.49	Octacosyl trifluoroacetate
35	26.464	978573	0.29	0.51	. gamma. -Sitosterol
		342626208	100.00	100.00	

Table 4.1. List of potential allelochemicals from methanolic extracts of *Prosopis juliflora*

Potential allelochemicals	Molecular formula	Reference
Octadecanoic Acid (21)	$C_{18}H_{36}O_2$	(30)
4-Vinylphenol (3)	C_8H_8O	
2-Methoxy-4-vinylphenol (4)	$C_9H_{10}O_2$	(31)
Squalene (33)	$C_{30}H_{50}$	
Neophytadiene (12)	$C_{20}H_{38}$	
Stigmasterol (32)	$C_{29}H_{48}O$	
Campesterol (30)	$C_{28}H_4$	(32)
Diethyl Phthalate (8)	$C_{12}H_{14}O_4$	
Alpha-Methyl Mannofuranoside (9 & 10)	$C_7H_{14}O_6$	

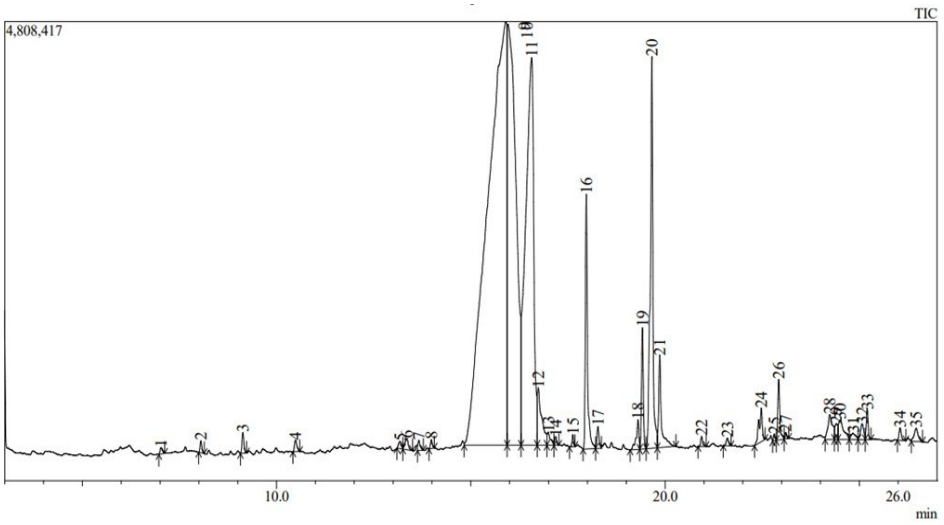

Fig. 2. The GC-MS chromatogram of methanolic extracts from *Prosopis juliflora*

Table 5. List of bioactive compounds present in GC-MS analysis of methanolic extracts of *Ocimum tenuiflorum*

Peak	R. Time	Area	Area percent	Height percent	Name of compounds
1	7.533	266523	0.25	0.23	N-Acetyl-3-pyrroline
2	8.051	253278	0.24	0.28	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-
3	8.541	735812	0.70	0.78	endo-Borneol
4	9.135	163162	0.16	0.17	4-Vinylphenol
5	9.206	425289	0.41	0.48	L-Proline, 1-acetyl-
6	11.042	268706	0.26	0.32	Phenol, 2-methoxy-3-(2-propenyl)-
7	11.433	221400	0.21	0.27	Copaene
8	11.650	47070190	44.84	51.00	Methyleugenol
9	12.037	7949498	7.57	9.17	Caryophyllene
10	12.151	747646	0.71	0.78	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4)
11	12.490	535496	0.51	0.53	Humulene
12	12.807	642881	0.61	0.64	Germacrene D
13	12.920	257424	0.25	0.23	Naphthalene, decahydro-4a-methyl-1-methyl
14	12.997	253023	0.24	0.24	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro
15	13.140	657657	0.63	0.70	Cyclohexane, 1-ethenyl-1 methyl-2,4-bis
16	13.975	196111	0.19	0.20	Diethyl Phthalate
17	14.060	985808	0.94	1.06	Caryophyllene oxide
18	14.937	605766	0.58	0.39	6-Ethoxy-6-methyl-2-cyclohexenone
19	15.684	429810	0.41	0.34	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxy
20	16.741	647123	0.62	0.56	Neophytadiene
21	17.626	206844	0.20	0.25	Hexadecanoic acid, methyl ester
22	17.967	5736338	5.46	5.78	n-Hexadecanoic acid
23	19.245	133161	0.13	0.16	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
24	19.303	364564	0.35	0.44	11,14,17-Eicosatrienoic acid, methyl ester
25	19.414	1428094	1.36	1.50	Phytol
26	19.654	13303129	12.67	10.01	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
27	19.860	1640018	1.56	1.49	Octadecanoic acid
28	20.329	891444	0.85	1.04	2-Hexadecen-1-ol,3,7,11,15-tetramethyl.
29	20.466	684461	0.65	0.68	Z-2-Octadecen-1-ol acetate
30	20.938	210101	0.20	0.25	Dimethyl aminoethyl palmitate
31	21.317	1214399	1.16	0.29	. alpha. -Amyrin
32	21.600	227132	0.22	0.25	Eicosanoic acid
33	22.405	355199	0.34	0.43	2-(Diethylamino)ethyl vaccenoate
34	23.660	1138803	1.08	0.61	Olean-12-en-3-ol, acetate, (3. beta.)-
35	24.227	145083	0.14	0.17	cis, cis, cis-7,10,13 Hexadecatrienal
36	24.503	1556795	1.48	0.83	Ergost-5-en-3-ol, (3. beta.)-
37	25.065	3624352	3.45	1.98	Stigmasterol
38	25.200	2393529	2.28	2.13	Squalene
39	26.453	5740319	5.47	3.00	. gamma. -Sitosterol
40	26.882	676450	0.64	0.36	1-Heptacosanol
		104982818	100.00	100.00	

Table 5.1. List of potential allelochemicals from methanolic extracts of *Ocimum tenuiflorum*

Potential allelochemicals	Molecular formula	Reference
Methyl eugenol	$C_{11}H_{14}O_2$	(33)
Caryophyllene	$C_{15}H_{24}$	(34)
Caryophyllene Oxide	$C_{15}H_{24}O$	
Humulene	$C_{15}H_{24}$	(35)
1-Heptacosanol	$C_{27}H_{56}O$	(29)
2-methoxy-3-(2-propenyl)- (Eugenol)	$C_{10}H_{12}O_2$	(36)
Copaene	$C_{15}H_{24}$	(37)

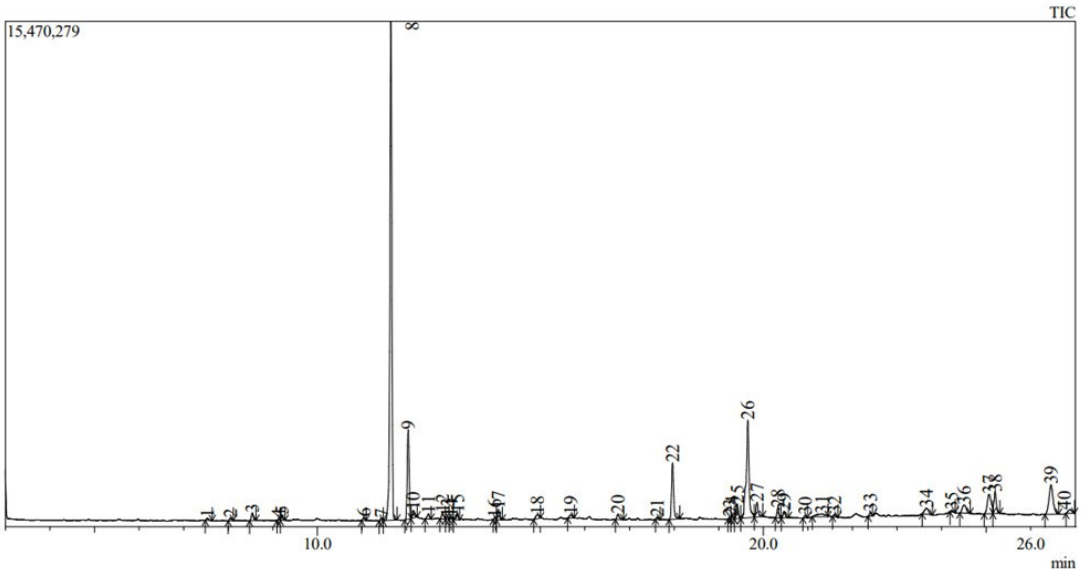

Fig. 3.The GC-MS chromatogram of methanolic extracts from *Ocimum tenuiflorum*

Table 6. Effects of formulated natural herbicides on weed density (No.m^{-2}) of *E. colona* at 15, 30 and 45 DAS

T.NO	15 DAS	30 DAS	45 DAS
T_1	3.75 (13.65)	2.35 (5.02)	6.98 (48.31)
T_2	3.72 (13.4)	2.32 (4.88)	7.00 (48.53)
T_3	3.61 (12.6)	2.2 (4.33)	6.82 (46.10)
T_4	4.26 (17.8)	2.52 (5.87)	7.39 (54.17)
T_5	4.08 (16.2)	2.41 (5.32)	7.16 (50.97)
T_6	3.86 (14.5)	2.32 (4.92)	7.08 (49.63)
T_7	3.06 (9.2)	2.18 (4.25)	6.28 (38.97)
T_8	2.97 (8.4)	2.26 (4.60)	6.29 (39.10)
T_9	1.97 (3.4)	1.74 (2.53)	6.24 (38.43)
T_{10}	1.44 (1.6)	1.23 (1.03)	1.52 (1.83)
T_{11}	4.79 (22.59)	6.13 (37.1)	8.31 (68.53)
S.Ed	**0.2230**	**0.0633**	**0.1835**
CD(P=0.05)	**0.4652**	**0.1320**	**0.3828**

Data were subjected to square root transformation. The value in parenthesis are original values.

Table 7. Effects of formulated natural herbicides on dry weight of *E. colona* (g m^{-2}) at 15, 30, 45 DAS

T.NO	15 DAS	30 DAS	45 DAS
T_1	2.06 (3.76)	1.93 (3.23)	6.06 (36.23)
T_2	2.04 (3.67)	2.10 (3.93)	6.08 (36.40)
T_3	1.94 (3.25)	1.93 (3.23)	5.92 (34.58)
T_4	2.22 (4.40)	2.06 (3.73)	6.22 (38.23)
T_5	2.12 (3.99)	2.12 (4.03)	6.41 (40.63)
T_6	2.05 (3.69)	1.90 (3.13)	6.14 (37.23)
T_7	1.92 (3.19)	2.03 (3.63)	5.45 (29.23)
T_8	1.99 (3.45)	2.01 (3.54)	5.46 (29.33)
T_9	1.55 (1.90)	1.57 (1.98)	5.42 (28.83)
T_{10}	1.13 (0.78)	1.13 (0.77)	1.37 (1.38)
T_{11}	5.33 (27.88)	5.26 (27.25)	7.20 (51.40)
S.Ed	**0.0555**	**0.1242**	**0.1581**
CD (P=0.05)	**0.1157**	**0.2591**	**0.3297**

Data were subjected to square root transformation and statistically analyzed the value in parenthesis are original values.

Table 8. Effects of formulated natural herbicides on weed control efficiency (%) of *E. colona* 15, 30, 45 DAS

T.NO	15 DAS	30 DAS	45 DAS
T_1	86.49	88.17	29.45
T_2	86.85	85.6	29.14
T_3	88.37	88.17	32.8
T_4	84.21	86.33	25.63
T_5	85.72	85.23	21.06
T_6	86.76	88.53	27.59
T_7	88.58	86.67	43.14
T_8	87.68	87.03	43
T_9	93.18	92.74	44
T_{10}	97.22	97.17	97.33
T_{11}	0	0	0

Grain yield and weed index

The results of grain yield of rice and the weed index indicated a significant difference between treatments that used methanolic extracts of *C. gigantea*, *Prosopis juliflora* and *O.tenuiflorum* combining with hand weeding at 25 DAS. In Table 9, a 10% *O. tenuiflorum* extract (T_9) produced the highest grain yield of 5393.33 kg ha^{-1}. The 10% methanolic extracts of *C. gigantea* (T_3) and *P. juliflora* (T_6) yielded significantly less grain producing 5183.33 kg ha^{-1} and 5070.00 kg ha^{-1}, respectively. The weed-free check (T_{10}) produced the highest yield of 5700.00 kg ha^{-1}, demonstrating the value of effective weed control. The weed-check produced the lowest yield (3543.33 kg ha^{-1}), demonstrating the detrimental effect of uncontrolled weed populations. The weed index also corresponded closely to grain yield (T_{10} = 0, T_{11} =37.9). In comparison to the other botanicals, the *O. tenuiflorum* 10% (T_9) extract was the most effective weed control treatment with an index of 5.55 indicating high phytotoxicity on *E. colona*. The lower dosages of *O. tenuiflorum* (T_7 and T_8) were also effective; however, effectiveness was notably lower than T_9. The high concentration of either *C. gigantea* (T_1, T_2) and *P. juliflora* (T_4, T_5) were also less effective overall and there continued to be more control with lower weed indices than the respective higher concentrations (Weed index= 9.95 & 10.95, respectively).

Economic Analysis

The EC formulation with 10% methanolic extract of *O. tenuiflorum* (T_9) recorded the highest net income value of ₹71,405 with the highest benefit cost ratio of 2.14 and was hence found to be economically more viable. Lower concentrations of *O. tenuiflorum*, represented as T_7 and T_8, also performed well, recording net incomes of ₹67,063.50 and ₹67,744 with benefit cost ratios of 2.08. The treatments with *C. gigantea*, namely T_1, T_2, and T_3 and *P. juliflora*, namely T_4, T_5, and T_6, resulted in lower net incomes, which ranged from ₹62,182.50 to ₹66,430 and benefit: cost (B:C) ratios ranging from 1.99 to 2.07. On the other hand, weed-free control (T_{10}), while resulting in complete eradication of weeds, had the highest cost of cultivation ₹70,765 a net income of ₹70,275.50, and maintained a B:C ratio of 1.99. The weedy control T_{11} resulted in the lowest net income of ₹29,161 and gave the least favorable benefit-cost ratio of 1.5 as evident from Table 10. Overall, *O. tenuiflorum* at 10% concentration proves to be the most effective and economical approach for *E. colona* management. Optimization of herbicide application rate considering cost-benefit ratio becomes prerequisite for profitability.

Table 9. Effects of formulated natural herbicides on grain yield (kg ha^{-1}) of rice and weed index

T.NO	Grain yield (kg ha^{-1})	Weed index
T_1	5081.67	10.46
T_2	5156.67	9.6
T_3	5183.33	9.2
T_4	5016.67	12.3
T_5	5066.67	11.42
T_6	5070.00	11.21
T_7	5216.67	8.76
T_8	5243.33	8.22
T_9	5393.33	5.55
T_{10}	5700.00	0
T_{11}	3543.33	37.9
SEd	**68.9614**	
CD(p=0.05)	**143.8512**	

Table 10. Effects of natural herbicides on economics of various weed management

T.NO	Cost of cultivation (₹)	Gross return (₹)	Net income (₹)	B:c ratio
T_1	62,365	128,170	65,805	2.06
T_2	62,365	128,223	65,858	2.06
T_3	62,365	128,795	66,430	2.07
T_4	62,365	124,547.50	62,182.50	1.99
T_5	62,365	125,688.50	63,323.50	2.02
T_6	62,365	125,966.50	63,601.50	2.02
T_7	62,365	129,428.50	67,063.50	2.08
T_8	62,365	130,109	67,744	2.08
T_9	62,365	133,770	71,405	2.14
T_{10}	70,765	141,040.50	70,275.50	1.99
T_{11}	58,788	87,949	29,161	1.5

Discussion

The findings of this study demonstrate the potential of methanolic extracts from *O. tenuiflorum*, *C. gigantea* and *P. juliflora* as natural herbicides for controlling *E. colona* in rice ecosystems. Among the tested botanicals, *O. tenuiflorum* emerged as the most effective way in terms of reducing weed density, dry weight and enhancing weed control efficiency, as well as positively influencing grain yield and economic returns of rice. These results align with previous studies that identified the herbicidal properties of allelochemicals found in plant extracts (19, 20).

Weed Flora Dynamics

The dominance of *E. colona* in the experimental fields, followed by *Cyperus difformis*, *Cyperus rotundus*, *Ludwigia parviflora* and *Panicum repens*, corroborates with the previous findings by Mandal *et al.* (2011) (21) and Mishra & Singh 2007 (22). Grasses like *E. colona* were observed to outcompete broad-leaved weeds and sedges, a trend that has significant implications for weed management strategies in rice ecosystems. The persistence of such species emphasizes the need for effective and targeted control measures to ensure minimal competition with the rice crop.

Allelochemicals and Phytotoxicity

The GC-MS analysis identified multiple bioactive compounds in the methanolic extracts of *O. tenuiflorum*, *C. gigantea* and *P. juliflora* that may possess allelopathic properties. The presence of fatty acids, phenolic compounds and terpenoids such as oleic acid, methyl eugenol and neophytadiene aligns with findings from Cui *et al.* (2012) (23), Li *et al.* (2010) (24) and Wang *et al.* (2008) (25) who reported these compounds as significant allelochemicals capable of inhibiting weed seed germination and growth. The phytotoxicity of these compounds, particularly in *O. tenuiflorum*, demonstrated significant suppression of *E. colona* at early growth stages. This supports earlier reports by Sharma & Singh (2004) (26) and Nongmaithem *et al.* (2012) (27) that natural plant extracts can be effectively utilized as herbicides.

Weed Density and Dry Weight

Ocimum tenuiflorum at 10% methanolic extract concentration (T_9) resulted in significantly lower weed density and dry weight compared to other treatments. The results showed a consistent reduction in weed population at 15 and 30 DAS, but a slight resurgence at 45 DAS, suggesting a declining herbicide efficacy over time. This drop in efficacy at later growth stages may indicate the need for supplemental weed management practices, such as additional hand weeding or higher concentrations of botanical extracts, to maintain control over *E. colona*. Similar observations were made by Islam & Kato-Noguchi. 2014 (28) when studying the inhibitory effects of methanolic extracts on barnyard grass.

Weed Control Efficiency

The results on weed control efficiency (WCE) reinforce the effectiveness of *O. tenuiflorum* in managing *E. colona*, especially at the 10% concentration. The WCE was highest at 15 and 30 DAS, with control efficiency exceeding 90%, but it decreased to 44% at 45 DAS, highlighting the potential for weed regrowth or reduction in herbicidal potency. In contrast, lower concentrations of *C. gigantea* and *P. juliflora* showed relatively poor control, which supports the hypothesis that higher concentrations of allelochemicals are necessary for achieving effective weed management (24, 25).

Practical Implications and Future Directions

The study highlights the potential for *O. tenuiflorum* to be developed into a natural herbicide for controlling *E. colona* in rice ecosystems. However, the observed decline in efficacy at 45 DAS suggests that further research is needed to optimize application timing and explore the use of combinations with other management strategies, such as mechanical weeding or integration with synthetic herbicides. Additionally, research into the formulation stability of these extracts over time and under varying environmental conditions could provide insights into improving their long-term efficacy. Moreover, while *C. gigantea* and *P. juliflora* were less effective in this study, their allelopathic potential should not be disregarded. Investigating different extraction methods, dosages and combinations of botanicals might reveal alternative strategies for natural weed control.

Conclusion

This research highlights the opportunity for a natural herbicide from plant extract to be used as a sustainable option for managing *E. colona* in rice ecosystem. Amongst the botanical's treatments, PE application of 50% EC formulations of 10% methanolic extract of *O. tenuiflorum* + one hand weeding at 25 DAS exhibited most effective reduction in weed density, dry weight and weed index and highest weed control efficiency, grain yield and benefit: cost ratio in the field experiments. However, some issues were realized regarding formulation stability and therefore, further optimization is essential to enhance the life of this natural herbicide. The integration of environment-friendly herbicides into rice production can lead to considerable reduction in the use of synthetic chemicals, thereby reducing environmental pollution and the development of resistance within weed populations. Future research would benefit from studying the natural solutions like those in this experiment and supplementing agronomic practices with more natural-based herbicides for short-term and long-term use in rice cultivated environments; additional research describing the potential effect or reduced synthetics may impact crop yield and ecology would complement these studies. This research reinforces the trend seeking sustainable agricultural practices and potentially a new natural herbicide or compounds for rice culture weed control.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Venture Capital Scheme of the Department of Agronomy, Agricultural College and Research Institute, Madurai, Tamil Nadu, India. We also extend our thanks to the DST-FIST (Department of Science and Technology - Fund for Improvement of S&T Infrastructure) scheme for providing infrastructural support, which was instrumental in the successful completion.

Authors' contributions

MMS, AG executed the field research, JP and PR few laboratory analyses, whereas ES and PA conceived the idea and supervised the work

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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