



# Synthesis And Structure Activity Relationships Of Substituted Cinnamic Acid Amide Derivatives: Herbicidal Activity

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**Abstract:** In this study, substituted cinnamic acids (4-hydroxy, 3-nitro, 4-nitro, 2-chloro, and 4-methoxy) and their amides were synthesised. The synthetic compounds were tested for seed germination inhibitory effectiveness on barnyardgrass (*Echinochola colona*) seeds at 50, 100, and 200 ppm concentrations, and the results were compared to standard herbicide formulations containing butachlor and pendimethalin. All substances displayed significant action. The majority of the chemicals exhibited limited inhibitory action at 50 ppm concentration. Most of the compounds had activity comparable to conventional butachlor and pendimethalin. Thus, substituted cinnamic acids and their amides may be produced as potential herbicides.

**Keywords:** Substituted cinnamic acids, cinnamic acid amide derivatives, barnyardgrass (*Echinochola colona*), butachlor, pendimethalin, Seed germination inhibition activity.

**Introduction:** It is evaluated that the cinnamic acid and substituted cinnamic acid have several biological properties such as antiviral, anti-inflammatory, antioxidant, antimicrobial and cell proliferation inhibitor enzyme. (1-3). Cinnamic acids and substituted cinnamic acid comes under aromatic carboxylic acids (C6-C3). Generally, those found in numerous amounts of plant species such as in coffee beans, olives, propolis, fruits, in many essential oils, resins, vegetables etc. Usually they are found as simple derivatives of esters, amides, sugar and glycosides.

A weed is a plant which competes with agricultural crop for their every requirement. The large amount of native and non native species are not required in a particular condition, "a plant in the wrong place"

There are many losses in entire world due to weeds. The discovery of herbicide 2, 4-dichlorophenoxy acetic acid (2, 4-D) by Zimmerman and Hitchcock, the agrochemical industry has successfully developed a wide range of herbicides with different chemical structures and modes of action (1). But indiscriminate and continuous use of herbicides has resulted in the occurrence of herbicide resistant weeds. According to one study, worldwide at least 15 weed biotypes, 194 weed species, including 114 dicots and 80 monocots have been reported to have acquired resistance to important herbicides (2).

Therefore, it is an unavoidable requirement to synthesise effective herbicide with the new structure and mode of action to reduce the problem of the weeds to herbicides. Less degradation of pesticides, constant of pesticides in soil and water for a long duration and pesticide residue problem in crops and vegetables, in view of environmental concerns, there has been a model in the pesticide development strategies. Recently allelopathy has been accepted as one of the best strategies for the development of pesticides.

Allelochemicals exist in plants act as a bio- communicators in nature. They are potential source of models of new structural types of herbicides (3, 4). During the past three decades, efforts have been focused on discovering new allelochemicals with potential application in weed management (5). The biocides developed from allelochemicals have important advantages with respect to traditional herbicides, fungicides or insecticides as they have novel modes of action, high biodegradability and low environmental persistence (6). There is an enormous diversity of allelochemicals in nature. Around 10,000 compounds are known, whereas another 4,00,000 are estimated to exist (7). Many plant species have been investigated and lots are under investigation for allelochemical studies. Recently cinnamic acid ester namely (E)-3-methoxyphenyl-4-hydroxycinnamate isolated from *Oxalis pes-caprae* L. (Oxalidaceae) was found to exhibit significant seed germination inhibition effect on *Lactuca salive* L (8).

The studies were carried out by synthesizing a large number of analogues of substituted cinnamic acids and amide derivatives and screening those for pesticidal activity. Very encouraging results were obtained and we came up with a new class of herbicides (9). In this paper we report herbicidal activity of substituted cinnamic acids and amide derivatives, It may be a new class of herbicides, against barnyardgrass an important weed of rice.

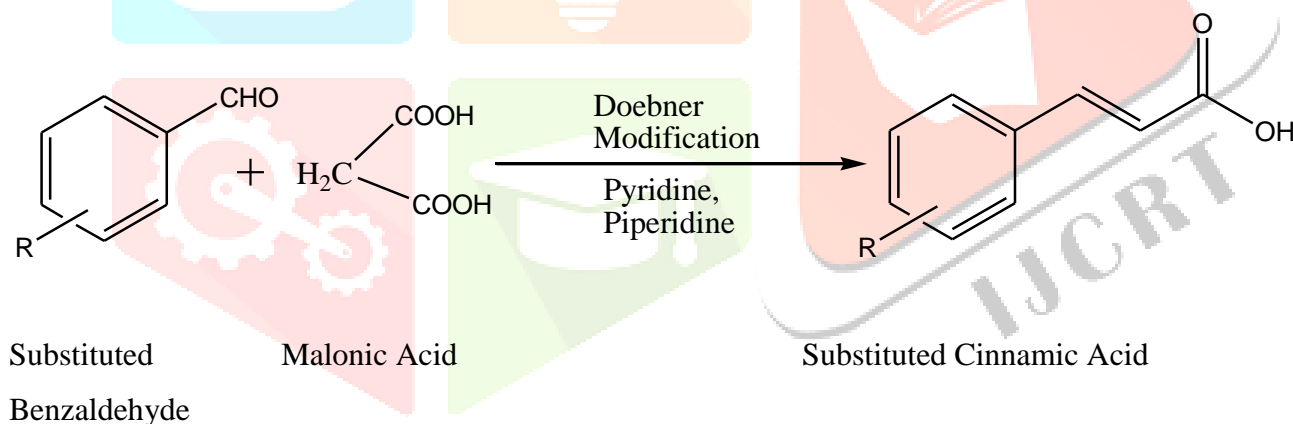
**General.** Melting points were determined on a Buchi B-540 apparatus and are uncorrected. UV spectra were taken on a Genesys 10 UV-vis spectrophotometer, <sup>1</sup>H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrophotometer, and IR spectra were recorded on a Bruker IR spectrophotometer.

The chemicals used in the present study, 4-hydroxy benzaldehyde, 3-nitrobenzaldehyde, 4-nitro benzaldehyde, 2-chlorobenzaldehyde, 4-methoxy benzaldehyde, malonic acid, piperidine, cyclohexylamine, aniline, N-methyl aniline, 2-chloro aniline, and morpholine, were purchased from M/S Sigma-Aldrich Chemical Co. (USA), and pyridine, triethylamine, and phosphorus oxychloride were purchased from M/S Merck India Chemicals Company.

Butacholor and pendimethalin were obtained from M/S Bayer AG, Germany. All of the chemicals were of AR grade. The barnyardgrass (*Echinochola colona*) seeds were obtained from Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, India.

**Preparation of Substituted Cinnamic Acids.** The substituted cinnamic acids were synthesized using Doebner-Knoevenagel modification (10). Cinnamic acids were prepared by mixing substituted benzaldehydes (60 mM), malonic acid (100 mM), pyridine (20 mL), and piperidine (0.8 mL) together and stirring on a magnetic stirrer for 4 days (Scheme 1). The reaction progress was monitored over silica gel TLC plates. The reaction mixture was then poured in excess of diluted HCl. Precipitates obtained were filtered, washed with cold water repeatedly, and dried. The yields were between 70-98% .

**Scheme1.** Synthesis of Substitued Cinnamic acid



Substituents:

R= Benzaldehyde

R= 4-nitro

R= 2-chloro

R= 4-hydroxy

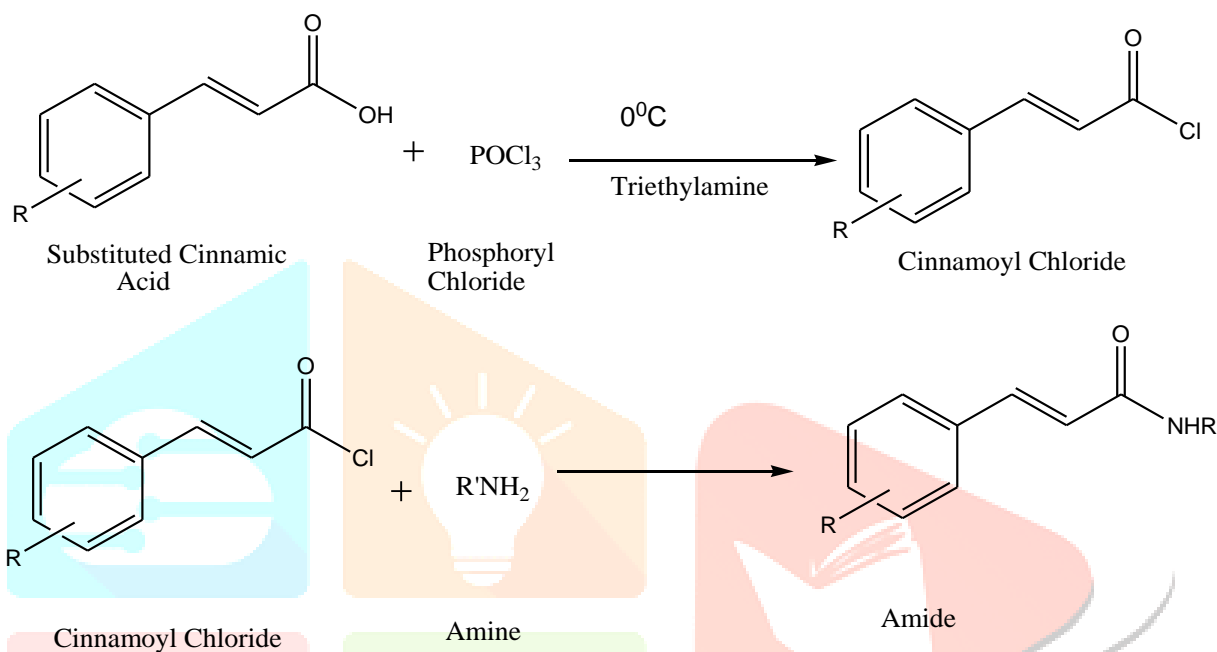
R= 3-nitro

R= 4-methoxy benzaldehyde

**Preparation of Substituted Cinnamic Acid Amide Analogues.** All of the amides were synthesized using the method described by Greenstein and Winitz (11). To a solution of acid (1.0 mM) in chloroform containing triethylamine (0.1 mL) and amino compound (1.2 mM) was added dropwise  $\text{POCl}_3$  (2.0 mM) at  $0^\circ\text{C}$  with constant stirring. Triethylamine (0.2 mM) was then added in one portion. The reaction mixture was stirred for another 30 min at low temperature and then poured into crushed ice. The organic phase separates out. The

aqueous phase was extracted with dichloromethane, and combined layers were washed successively with diluted HCl, aqueous NaHCO<sub>3</sub>, water and dried over anhydrous CaCl<sub>2</sub> (**Scheme 2**). Evaporation of solvent gave residues of varying colors, which were recrystallized from ethanol/chloroform (80:20). The yields were between 75-92%

### Scheme 2. Synthesis of amides of substituted Cinnamic Acids



**Bioassay.** A bioassay based on seed germination was used to study seed germination inhibition activity of the synthesized compounds. Test solutions of concentration 50 ppm, 100 ppm, and 200 ppm were prepared by serial dilution of stock solution of 500 ppm of the compounds in ethanol. The test solutions of standard butachlor and pendymethylene were also prepared by serial dilution in distilled water. Each compound was dissolved in a minimum amount of ethanol, which was suspended in distilled water with Tween-20 (3 mL/l). Seeds of barnyardgrass (*Echinochola colona*) prior to sowing in Petri dishes (9 cm diameter) were surface sterilized with distilled water followed by 95% ethanol for 15 s and finally with distilled water.

Twenty seeds were taken in each Petri dish. Seed germination paper was placed in the Petri dishes. In each petri dish 7 mL of each test solution was poured. A mixture of distilled water/ ethyl alcohol/Tween-20 (97: 2.5: 0.5) was taken as the control. All treatments were replicated three times with CRD fashion. The barnyardgrass (important weed of rice) seeds were allowed to germinate at 35°C. The seed germination process was observed after five days of incubation and seeds were considered to be germinated when the protrusion of radical was evident (**12**, **13**). After 125 h, the number of seeds germinated in each Petri dish was checked and counted. After the calculation of germinated seeds, percent seed germination inhibition values were calculated.

**Statistical Analysis.** The percent seed germination inhibition values were determined and subjected to analysis of variance (ANOVA). Critical differences (CDs) were calculated at  $P= 0.05$ .

## RESULTS AND DISCUSSION

Persual of **Table-1** clearly indicates that there is not significant increase in mean percent seed germination inhibition (SGI) activity with increase in concentration except for 4-methoxy cinnamoyl piperidine. Thus in most of the cases activities are at par for different concentration. At 50 ppm conc. highest SGI activity is exhibited by cinnamoyl piperidine. Values of other compounds except 4-hydroxy cinnamoyl piperidine and butachlor (standard) including pendymethylene (standard) are par with that of cinnamoyl piperidine. Lower activity for 4-hydroxy cinnamoyl piperidine may be attributed to the presence of electron donating -OH substituent group present on benzene ring of acid moiety of amide.

At 100 ppm concentration highest SGI activity is observed again for cinnamoyl piperidine and the values is at par with 2- chloro cinnamoyl piperidine. Values of other compounds even including standard Butachlor and pendymethylene are significantly lower than cinnamoyl piperidine, this may be attributed to the presence of electron withdrawing chlorine group present on benzene ring on acid moiety. One more factor seems responsible for effecting the activity less bulky group are more effective in increasing or decreasing activity. Less bulky chlorine group are amongst electron attracting group tends to increase the activity more in comparison to more bulky group ( $\text{NO}_2$ ) likewise less bulky electron donating group (-OH) is more effective in comparison to more bulky (-OCH<sub>3</sub>) group in decreasing the activity. At 200 ppm concentration the same trend is observed.

**Table1.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of piperidine

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
Cinnamoyl piperidine	66.66	71.66	75.00	9.40
1-(2'-chlorocinnamoyl) piperidine	58.33	63.33	71.66	16.29
1-(4'-methoxy cinnamoyl) piperidine	55.00	61.67	70.00	8.79
1-(3'-nitrocinnamoyl) piperidine	55.00	58.33	63.33	14.87
1-(4'-nitrocinnamoyl) piperidine	56.66	58.33	61.66	11.51
1-(4'-hydroxy cinnamoyl) piperidine	53.33	58.33	63.33	5.75
Cd at 5%	12.98	9.34	7.28	

Persual of Table-2 reveals that for most of the compounds mean percent SGI activity do not increase significantly with increase in concentration. At 50 ppm concentration maximum value for mean percent (SGI) activity is obtained is exhibited by N cyclohexyl(1-(4-NO<sub>2</sub>)cinnamamide which is at par with N-cyclohexyl cinnamamide. Other compounds (N-cyclohexyl 1-(2'-chlro) cinnamamide, N-cyclohexyl-1-(4'-methoxy) cinnamamide and N-cyclohexyl-1-(3'-nitro) cinnamamide)) including standard butachlor and pendymethylene exhibit lower (SGI) activity.

Higher value in case of N cyclohexyl(1-(4-NO<sub>2</sub>)cinnamamide may be attributed to presence of electron attracting NO<sub>2</sub> group though effect of substituted group is not contrasting in case of other compounds. At 100 ppm same trend is observed.

At 200 ppm concentration highest values exhibited again by N cyclohexyl(1-(4-NO<sub>2</sub>)cinnamamide and values of all other compounds are at par except that of N cyclohexyl(1-(3-NO<sub>2</sub>)cinnamamide.

**Table 2.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of cyclohexyl amine.

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
N-cyclohexyl cinnamamide	65.00	66.67	70.00	6.65
N-cyclohexyl 1-(2'-chlro) cinnamamide	53.33	55.00	68.33	11.02
N-cyclohexyl-1-(4'-methoxy) cinnamamide	53.33	56.67	65.00	7.43
N-cyclohexyl-1-(3'-nitro) cinnamamide	53.33	58.33	58.33	15.23
N-cyclohexyl-1-(4'-nitro) cinnamamide	71.66	73.33	73.33	16.29
Cd at 5%	12.38	8.54	10.10	

Persual of table-3 indicates that for all the compounds mean percent (SGI) activity do not increases significantly with increase in concentration from 100 ppm to 200 ppm. For all compounds values at 100 ppm concentration are at par with value of 200 ppm.

At 50 ppm concentration maximum value for mean percent (SGI) activity is exhibited by 4-Nitrocinnamanilide which is at par with cinnamanilide and 4-hydroxy-cinnamanilide. Other comopounds (2-chlro cinnamanilide, 4-methoxy cinnamanilide, 3-nitro cinnamanilide) including butachlor and pendymethylene exhibit lower mean percent (SGI) activity. Higher value in case of 4-nitro cinnamanilide may be attributed to presence of electron attracting nitro group though effect of substituent groups for other compounds could not be unable with electron donating or electron attracting tendencies of substituents groups.

At 100 ppm concentration highest mean percent (SGI) activity is observed again for 4-nitrocinnamanilide which is at par with all other compounds including standards. The case of 3-nitrocinnamanilide is exceptional as it exhibit lower mean percent (SGI) activity inspite of having electron withdrawing substituent group.

At 200 ppm concentration mean percent (SGI) activity is observed again for 4-nitrocinnamanilide which is at par with the compounds (cinnamanilide, 4-methoxy cinnamanilide, 4-nitro cinnamanilide, 4-hydroxy cinnamanilide) compounds including standard exhibiting significantly lower mean percent (SGI) activity.



**Table 3.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of aniline

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
Cinnamanilide	61.67	71.67	76.67	5.75
2 chlorocinnamanilide	55.00	60.00	70.00	12.87
4-methoxy cinnamanilide	55.00	68.33	73.33	13.71
3-nitro cinnamanilide	58.33	58.33	68.33	15.23
4-nitro cinnamanilide	71.67	71.67	76.67	5.75
4-hydroxy cinnamanilide	60.00	66.67	71.67	9.40
Cd at 5%	12.11	9.17	7.49	

Table-4 indicate that there is no significant increase in Mean Percent Inhibition value with increase in concentration for compounds (N- methyl cinnamanilide, N- methyl-3-nitro cinnamanilide and N- methyl-4-nitro cinnamanilide). For compounds 1,4,5 there is significant increase in Mean Percent Inhibition value with increase in concentration.

At 50 ppm concentration highest mean percent (SGI) activity is exhibited by N-methyl cinnamanilide. Activities of other compounds including pendymethylene & butachlor are significantly lower than that of N-methyl cinnamanilide.

At 100 ppm concentration again highest mean percent (SGI) activity is exhibited by N-methyl cinnamanilide other compounds including standards butachlor and pendymethylene exhibit significantly lower mean percent (SGI) activity than N-methyl cinnamanilide.

At 200 ppm concentration same trend is observed. It is observed that any substituent whether electron withdrawing or electron donating present on benzene ring of acid moiety tends to decrease the activity.



**Table 4.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of N-methyl aniline

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
N- methyl cinnamanilide	70.00	75.00	85.00	11.51
N- methyl-1(2'-chlro )cinnamanilide	53.33	56.67	68.33	8.14
N- methyl-4-methoxy cinnamanilide	51.67	56.67	68.33	8.14
N- methyl-3-nitro cinnamanilide	53.33	53.33	56.67	5.75
N- methyl-4-nitro cinnamanilide	51.67	51.67	55.00	9.40
Cd at 5%	11.47	7.87	5.05	

Table-5 indicates that for compounds (Cinnamoyl morpholine, 1-(3'-nitro cinnamoyl) morpholine, 1-(4'-nitro cinnamoyl )morpholine ) there is no significant increase in mean percent (SGI) activity with oncrease in concentration whereas for compound (1,4,5) there is significant increase in Meen Percent Inhibition value with increase in concentration . At 50 ppm concentration 3-Nitro cinnamoyl morpholine and cinnamoyl morpholine exhibit highest activity. All other compounds including butachlor and pendymethylene (standard) are at par with cinnamoyl morpholine and 3-nitro cinnamoyl morpholine. At 100 ppm and 200 ppm sametrand is observed.

**Table 5.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of morpholine

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
Cinnamoyl morpholine	63.33	66.67	73.33	16.29
1-(2'-chlorocinnamoyl) morpholine	55.00	58.33	70.00	14.49
1-(4'-methoxy cinnamoyl) morpholine	56.67	66.67	76.67	16.29
1-(3'-nitro cinnamoyl) morpholine	63.33	63.33	66.67	11.51
1-(4'-nitro cinnamoyl )morpholine	56.67	61.67	61.67	25.10

Cd at 5%	15.16	13.23	11.93	
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Table-6 indicates that there is no significant increase in mean percent (SGI) activity with increase in concentration. Except for 2-chloro(2-chloro) cinnamanilide and butachlor. At 50 ppm concentration 2-chloro-4-nitro cinnamanilide shows highest activity. Other compounds including butachlor and pendymethylene are at par with it. 2-chloro (2-chloro) cinnamanilide and 2-chloro3-nitro cinnamanilide exhibit significantly lower activity than 2-chloro-4-nitro cinnamanilide. At 100 ppm same trend is observed. At 200 ppm concentration again 2-chloro-4-nitro cinnamanilide exhibit highest activity compounds (Cinnamoyl morpholine, 1-(2'-chlorocinnamoyl) morpholine) are at par with it. whereas compounds (2-chloro(4'-methoxy)cinnamanilide, 2-chloro-(3'-nitro)cinnamanilide, 2-chloro-(4'-nitro)cinnamanilide) exhibit significantly lower mean percent (SGI) activity the 2-chloro-4-nitro cinnamanilide.

**Table 6.** Mean Percent Seed Germination Inhibition Values of Substituted Amides of 2-chloro aniline

Name of the Compound	Mean 50ppm	Percent 100ppm	Inhibition 200ppm	Value Cd at 5%
Butachlor (standard)	53.33	60.00	65.00	8.79
Pendymethylene (standard)	56.67	61.67	68.33	14.10
2- chloro cinnamanilide	60.00	68.33	73.33	14.87
2- chloro-(2'- chloro )cinnamanilide	50.00	53.33	75.00	11.98
2- chloro (4'- methoxy )cinnamanilide	56.67	60.00	68.33	13.71
2- chloro-(3'- nitro)cinnamanilide	50.00	53.33	65.00	15.59
2- chloro-(4'-nitro )cinnamanilide	63.33	71.67	78.33	17.27
2- chloro-(4'- hydroxy) cinnamanilide	56.67	63.33	68.33	15.23
Cd at 5%	12.11	14.88	9.00	

### Analytical and Spectral Characterization Data.

**1. 4-Methoxy Cinnamic Acid:** M.P. 174 °C ; yield 78.4%.  $\lambda_{max}$  (nm)/DCM, 304. IR  $\nu_{max}$  (cm<sup>-1</sup>, KBr): 3400-3300, 3110-3075, 2937, 1686.9, 1622.8, 1596, 1476.5, 1414.6, 1315.4. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) 6.28 (1H, d, *J*) 16 Hz, -CHdCH-, 7.57 (1H, d, *J*) 16 Hz, -CHdCH-, 3.81 (3H, s), 7.48 (2H, Ar, d, *J*) 13 Hz, H-3, H-5), 6.90 (2H, Ar, d, *J*) 14.5 Hz, H-2, H-6).

**2. 4-Hydroxy Cinnamic Acid:** M.P. 182 °C; yield 78.8%.  $\lambda_{\text{max}}(\text{nm})/\text{DCM}$ , IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>, KBr): 3378, 1675.2, 1630, 1598, 14446, 1212 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  7.6 (1 H, d, J= 15.9 Hz, H-3), 7.38 (2H, d, J=8.4 Hz, H-5 and H-8), 6.83 (2H, d, J=8.4 Hz, H-6 AND H-8), 6.25 (1H, d, J=15.9Hz, H-2).

**3. 3-Nitro Cinnamic Acid:** M.P. 194 °C; yield 96.6%.  $\lambda_{\text{max}}(\text{nm})/\text{DCM}$ , IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>, KBr): 3427, 1687, 1627, 1600, 1534, 1347, 845 cm<sup>-1</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  6.19 (1 H, d, J= 16.2 Hz, H-2), 7.28 (2H, d, J=16.2 Hz, H-5 and H-3), 7.84 (2H, d, J=8.7 Hz, H-5 and H-7), 7.35 (2H, d, J=8 Hz, H-8 and H-9).

## ACKNOWLEDGMENT

**Supporting Information Available:** Synthesis of substituted cinnamic acids, substituted cinnamic acids with aniline, substituted cinnamic acids with *o*-chloro aniline, substituted cinnamic acids with *p*-nitro aniline, substituted cinnamic acids with *p*-methyl aniline, and structures of substituted cinnamic acids, substituted cinnamanilides, substituted 2-chloro cinnamanilides, substituted 4-nitro cinnamanilides, and substituted 4-methyl cinnamanilides. The authors are thankful to the Dean, College of Basic Sciences and Humanities and Director Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar.

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