



## Antinematic activity of ferrocenyl chalcones against *Meloidogyne incognita* infestation in tomato

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### ABSTRACT

A series of ferrocenyl chalcones using acetylferrocene with different aldehydes were synthesized and their *in vitro* evaluation against *Meloidogyne incognita* was reported. The present study was carried out in net house conditions, Division of Nematology, ICAR-IARI, during *rabi* 2018–19, the bioefficacy evaluation of most effective compounds, identified by *in vitro* results, along with Carbofuran 3G in pot cultured tomato is reported. The identified, five most effective compounds, (2*E*)-1-Ferrocenyl-3-(4-bromophenyl)-prop-2-en-1-one (3b), (2*E*)-1-Ferrocenyl-3-(4-methoxyphenyl)-prop-2-en-1-one (3f), (2*E*)-1-Ferrocenyl-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one (3h), (2*E*)-1-Ferrocenyl-3-(4-nitrophenyl) prop-2-en-1-one (3m) and (2*E*)-1-Ferrocenyl-3-(2,6-dichlorophenyl)-prop-2-en-1-one (3o) were undertaken for pot experiment at different concentrations, viz. 20, 40 and 80 ppm and by soil drenching and root dipping methods. The study revealed that all plant growth parameters were positively influenced by these compounds. It was also observed that the presence of electron releasing group positively influences the efficacy and the activity was highest in compound 3f and 3h @ 80 ppm at 30 DAI and 60 DAI. The activity was higher in soil drenching compared to root dipping method.

**Keywords:** Ferrocenyl chalcones, *Meloidogyne incognita*, Root dipping, Root-knot nematode, Soil drenching

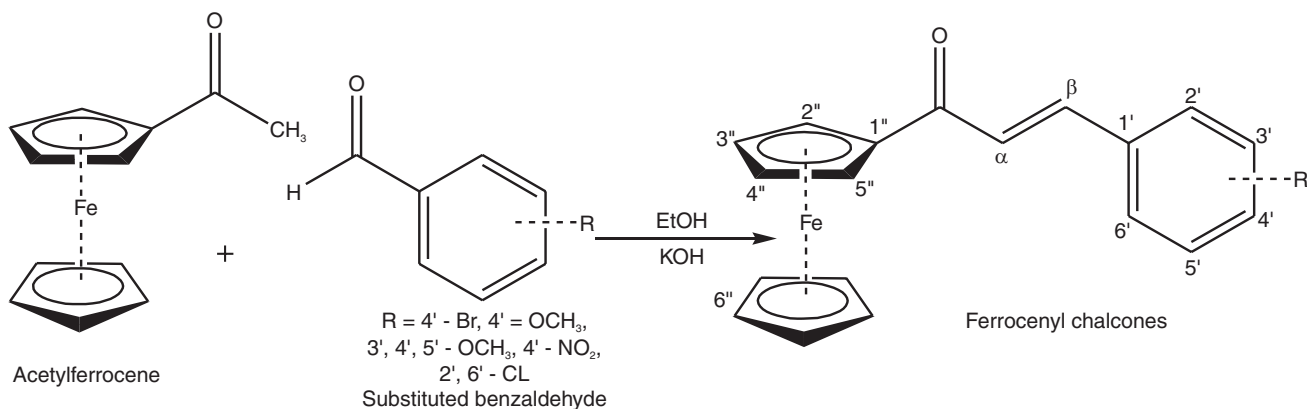
Chalcones and their corresponding heterocyclic analogs were reported to exhibit several biological activities due to presence of highly reactive unsaturated carbonyl moiety in skeleton (Jayasinghe *et al.* 2004, Kromann *et al.* 2004, Nielsen *et al.* 2005, Bag *et al.* 2009, Kalirajan *et al.* 2009, Shakil *et al.* 2011, 2013). Research is directed towards the development of potent crop protection chemicals based on this nucleus. However, poor solubility is a major concern and prevents their use in crop protection. In order to overcome this, efforts have been made to improve the solubility of substituted chalcones by the introduction of ferrocene group. Among various vegetable crops, Tomato (*Solanum lycopersicum* L. Solanaceae) is the second topmost remunerable crop (Anonymous, NHB, DAC&FW 2011). Annual tomato production of India contributes to 11.35% of the total world production (NHB database, DAC&FW, 2018). There are several diseases of tomato caused by fungi, bacteria, viruses, nematodes and abiotic factors (Abada *et al.* 2008; Zhou *et al.* 2016).

Plant parasitic nematodes (PPNs) are most important soil borne pests that cause damage to almost all species of crop plants (Kumar *et al.* 2020). Root-knot nematodes (RKNs), are the most destructive pathogens in terms of yield loss due to their broad host range, i.e. vegetables, cereals and pulses etc. (Abad *et al.* 2008, Liu *et al.* 2020). *Meloidogyne incognita* is one of the important pests of vegetables belonging to Solanaceous crops. There is practically no synthetic nematicide available till date. Some insecticides such as carbofuran have been used for nematode control. However, there is a question mark about carbofuran due to environmental concern (Mishra *et al.* 2020). The need of the present is to develop environmentally safe alternative for effective nematode control. Several natural and synthetic derivatives have been found effective against *M. incognita* (Shakil *et al.* 2008, Bose *et al.* 2010, Attar *et al.* 2011, Kaushik *et al.* 2015, 2019, Caboni *et al.* 2016, Yadav *et al.* 2019, 2020). In the present study, the bioefficacy evaluation of most potent molecules, identified on the basis of *in vitro* results, is reported in pot cultured tomato cv. Pusa Ruby under net house conditions.

### MATERIALS AND METHODS

*Synthesis and characterization:* A series of ferrocenyl chalcone have been synthesized by acetylferrocene and

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Scheme I - General method for the synthesis of ferrocenylchalcone

KOH were dissolved in ethanol in a round bottom flask and stirred at room temperature for 10 min, followed by drop wise addition of ethanolic solution of equimolar amounts of different benzaldehydes with continuous stirring. The stirring ranged between 1 to 40 h for different reactions at room temperature (Scheme-I). Characterization of synthesized compounds was done by various spectroscopic techniques, viz. IR, HR-MS, <sup>1</sup>H NMR and <sup>13</sup>C NMR (Yadav *et al.* 2019).

*In vivo nematocidal activity:* The soil required for *in vivo* experiment was collected from research farms of the ICAR-IARI, New Delhi. The soil was mixed with sand in the ratio of 3:1 and sieved through 20 mesh sieve, so that

big size clods and debris are removed. Further the soil was sterilized and thoroughly blended before use. Each pot was filled with 2 kg of sterilized soil. Twenty-five days old seedlings of tomato (cv. Pusa Ruby), grown in nursery beds, were transplanted into in small earthen pots of 10 cm × 10 cm. Treatments given as per the plan of experiment mentioned below. Pots were regularly watered till the end of the experiment. Based on *in vitro* results, five most effective compounds (2*E*)-1-Ferrocenyl-3-(4-bromophenyl)-prop-2-en-1-one (3b), (2*E*)-1-Ferrocenyl-3-(4-methoxyphenyl)-prop-2-en-1-one (3f), (2*E*)-1-Ferrocenyl-3-(3,4,5-trimethoxyphenyl)-prop-2-en-1-one

Table 1 Evaluation of test compounds on plant parameters of tomato infested with *M. incognita* after 60 DAI as soil drenching and root dipping

Test compound	Dose (ppm)	Soil drenching				Root dipping			
		Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh Root weight (g)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh shoot weight (g)
3b	80	42.57 <sup>bcd</sup>	23.43 <sup>a</sup>	11.70 <sup>abcde</sup>	3.70 <sup>abc</sup>	36.17 <sup>bc</sup>	19.67 <sup>ab</sup>	9.34 <sup>bc</sup>	3.53 <sup>ab</sup>
	40	40.90 <sup>de</sup>	18.87 <sup>bc</sup>	9.87 <sup>efghi</sup>	3.03 <sup>bcdef</sup>	31.53 <sup>ef</sup>	16.10 <sup>c</sup>	8.46 <sup>c</sup>	3.06 <sup>bcd</sup>
	20	33.50 <sup>hi</sup>	17.47 <sup>c</sup>	9.65 <sup>fghi</sup>	2.96 <sup>cdef</sup>	27.67 <sup>hi</sup>	13.67 <sup>e</sup>	6.86 <sup>def</sup>	2.34 <sup>g</sup>
3f	80	44.77 <sup>ab</sup>	24.07 <sup>a</sup>	12.26 <sup>abc</sup>	3.75 <sup>ab</sup>	38.50 <sup>ab</sup>	21.10 <sup>a</sup>	10.05 <sup>ab</sup>	3.61 <sup>a</sup>
	40	40.30 <sup>de</sup>	18.93 <sup>bc</sup>	11.84 <sup>abcde</sup>	2.88 <sup>def</sup>	37.13 <sup>abc</sup>	20.50 <sup>a</sup>	8.91 <sup>c</sup>	3.01 <sup>cde</sup>
	20	35.90 <sup>fg</sup>	15.67 <sup>c</sup>	10.14 <sup>defgh</sup>	2.64 <sup>def</sup>	30.67 <sup>fgh</sup>	14.37 <sup>cde</sup>	6.85 <sup>def</sup>	2.51 <sup>efg</sup>
3h	80	43.43 <sup>abc</sup>	23.93 <sup>a</sup>	12.51 <sup>ab</sup>	3.89 <sup>a</sup>	38.37 <sup>ab</sup>	20.43 <sup>a</sup>	9.98 <sup>ab</sup>	3.47 <sup>abc</sup>
	40	40.37 <sup>de</sup>	22.13 <sup>ab</sup>	12.12 <sup>abcd</sup>	3.33 <sup>abcd</sup>	35.30 <sup>bcd</sup>	18.60 <sup>b</sup>	7.40 <sup>d</sup>	3.06 <sup>bcd</sup>
	20	36.80 <sup>f</sup>	17.50 <sup>c</sup>	7.90 <sup>ij</sup>	3.00 <sup>bcdef</sup>	32.30 <sup>def</sup>	15.53 <sup>cd</sup>	7.22 <sup>de</sup>	2.42 <sup>fg</sup>
3m	80	40.27 <sup>de</sup>	21.43 <sup>ab</sup>	10.58 <sup>bcdefg</sup>	3.28 <sup>abcde</sup>	34.70 <sup>cde</sup>	19.67 <sup>ab</sup>	9.14 <sup>bc</sup>	3.63 <sup>a</sup>
	40	37.63 <sup>ef</sup>	17.27 <sup>c</sup>	10.24 <sup>cdefgh</sup>	3.04 <sup>bcdef</sup>	30.57 <sup>fgh</sup>	18.43 <sup>b</sup>	7.22 <sup>de</sup>	2.98 <sup>cde</sup>
	20	32.83 <sup>hi</sup>	15.73 <sup>c</sup>	8.37 <sup>hij</sup>	2.66 <sup>def</sup>	28.10 <sup>ghi</sup>	14.27 <sup>de</sup>	6.43 <sup>ef</sup>	2.56 <sup>defg</sup>
3o	80	42.57 <sup>bcd</sup>	21.57 <sup>ab</sup>	11.57 <sup>abcdef</sup>	3.33 <sup>abcd</sup>	31.00 <sup>fg</sup>	20.10 <sup>ab</sup>	9.48 <sup>abc</sup>	3.53 <sup>ab</sup>
	40	33.03 <sup>hi</sup>	17.83 <sup>c</sup>	10.63 <sup>bcdef</sup>	3.29 <sup>abcde</sup>	30.97 <sup>fgh</sup>	18.47 <sup>b</sup>	7.33 <sup>de</sup>	2.92 <sup>def</sup>
	20	30.57 <sup>i</sup>	17.60 <sup>c</sup>	8.57 <sup>ghij</sup>	2.55 <sup>ef</sup>	25.07 <sup>i</sup>	14.33 <sup>de</sup>	7.04 <sup>def</sup>	2.37 <sup>g</sup>
Carbofuran		45.43 <sup>a</sup>	24.10 <sup>a</sup>	12.94 <sup>a</sup>	3.91 <sup>a</sup>	39.60 <sup>a</sup>	21.20 <sup>a</sup>	10.52 <sup>a</sup>	3.77 <sup>a</sup>
Control		23.27 <sup>j</sup>	15.57 <sup>c</sup>	7.59 <sup>j</sup>	2.46 <sup>ef</sup>	19.73 <sup>j</sup>	13.23 <sup>e</sup>	6.41 <sup>ef</sup>	2.26 <sup>g</sup>
CD at 5%		2.79	3.50	2.03	0.75	3.32	1.74	1.05	0.51

(Mean of 3 replications)

No significant difference in mean with the same letters

(3h), (2*E*)-1-Ferrocenyl-3-(4-nitrophenyl)prop-2-en-1-one (3m) and (2*E*)-1-Ferrocenyl-3-(2,6-dichlorophenyl)prop-2-en-1-one (3o) were taken for pot experiment. The root-knot nematode, *M. incognita* population used in the experiments was isolated from the root galls of infected plants collected from the glass house of Division of Nematology, ICAR-IARI, New Delhi, India.

*Experiment no. 1: Seedling root dip:* About 25 days old seedlings were taken and subjected to seedling dip treatment at different concentrations, viz. 20, 40 and 80 ppm of developed effective compounds. Immediately after seedling dip (20 min.), about 1 seedling was transplanted into each 5" pot. After 48 h of transplanting, each pot was inoculated by freshly hatched J2s of *M. incognita* at the rate of 3 J2s /cc of soil.

*Experiment no. 2: Soil drenching:* Each pot was subjected to soil drenching treatment with different concentrations, viz. 20, 40 and 80 ppm of developed effective compounds. After 1 day, 25 days old seedlings were transplanted at 1 seedlings / pot (5"). After 48 hrs of transplanting, each pot was inoculated by freshly hatched J2s of *M. incognita* at the rate of 3J2s / cc of soil.

All the treatments were replicated 3 times and untreated inoculated, which served as negative control and commercial formulation of Carbofuran 3G, which served as positive control. The plants were uprooted at 30 and 60 days after

inoculation (DAI). The experimental plants were safely depotted by slightly watering the pots. Whole plant, along with soil, was put in a bowl containing tap water and the plant was taken out gently. The roots were washed thoroughly under tap water again and packed individually in polythene bags to avoid transpiration losses. Data on shoot length (cm), fresh shoot weight (g), root length (cm), fresh root weight (g) and number of galls per plant were recorded. One-way analysis of variance (ANOVA) was computed using online facility of Web Agri Stat Package (WASP 2.0) provided by ICAR-CCARI, Ela Old Goa.

## RESULTS AND DISCUSSION

The data (Table 1) showed that at 60 DAI as soil drenching, there was a significant raise in shoot length, shoot weight, root length and root weight in comparison to control. The shoot length was found highest (44.77 cm) in plants treated with compound 3f followed by 3h> 3b= 3o> 3m @ 80 ppm at 60 DAI. Plants treated with 3f showed maximum (24.07 cm) root length followed by 3h> 3b> 3o> 3m @ 80 ppm at 60 DAI. The fresh shoot weight was found highest (12.51 g) in plants treated with 3h followed by 3f> 3b> 3o> 3m @ 80 ppm at 60 DAI. Plants treated with 3h showed highest (3.89 g) fresh root weight followed by 3f> 3b> 3o> 3m @ 80 ppm at 60 DAI. The data (Table 1) showed that at 60 DAI as root dipping, there was a significant increase in

Table 2 Evaluation of test compounds on gall formation and yield of tomato infested with *M. incognita* as soil drenching and root dipping

Test compound		Average no. of galls/plant				Yield (g)	
Compound	Dose (ppm)	Soil drenching (SD)		Root dipping (RD)		SD	RD
		30 DAI	60 DAI	30 DAI	60 DAI		
3b	80	6.00 <sup>gh</sup>	9.00 <sup>ijk</sup>	10.00 <sup>gh</sup>	14.33 <sup>d</sup>	101.33 <sup>b</sup>	95.00 <sup>bc</sup>
	40	11.00 <sup>de</sup>	17.00 <sup>g</sup>	17.67 <sup>de</sup>	23.67 <sup>c</sup>	81.00 <sup>de</sup>	79.00 <sup>e</sup>
	20	21.33 <sup>b</sup>	32.33 <sup>bc</sup>	27.33 <sup>ab</sup>	39.00 <sup>ab</sup>	41.33 <sup>ijk</sup>	43.33 <sup>hi</sup>
3f	80	4.00 <sup>h</sup>	8.00 <sup>jk</sup>	9.67 <sup>gh</sup>	12.67 <sup>d</sup>	124.33 <sup>a</sup>	112.67 <sup>cd</sup>
	40	9.00 <sup>ef</sup>	15.67 <sup>gh</sup>	16.67 <sup>ef</sup>	21.67 <sup>c</sup>	89.33 <sup>cd</sup>	88.00 <sup>cd</sup>
	20	20.00 <sup>b</sup>	27.33 <sup>de</sup>	21.33 <sup>cd</sup>	36.67 <sup>b</sup>	52.00 <sup>hi</sup>	48.33 <sup>gh</sup>
3h	80	4.33 <sup>h</sup>	8.33 <sup>jk</sup>	10.00 <sup>gh</sup>	13.00 <sup>d</sup>	109.33 <sup>b</sup>	101.33 <sup>b</sup>
	40	9.67 <sup>ef</sup>	16.33 <sup>g</sup>	17.00 <sup>ef</sup>	21.33 <sup>c</sup>	84.33 <sup>d</sup>	85.67 <sup>de</sup>
	20	19.33 <sup>b</sup>	29.00 <sup>cd</sup>	23.67 <sup>bc</sup>	37.00 <sup>b</sup>	59.00 <sup>gh</sup>	54.00 <sup>g</sup>
3m	80	7.67 <sup>fg</sup>	11.00 <sup>ij</sup>	13.33 <sup>fg</sup>	14.67 <sup>d</sup>	100.33 <sup>bc</sup>	99.00 <sup>b</sup>
	40	12.67 <sup>cd</sup>	24.00 <sup>ef</sup>	21.00 <sup>cd</sup>	25.00 <sup>c</sup>	64.00 <sup>fg</sup>	67.33 <sup>f</sup>
	20	25.00 <sup>a</sup>	35.67 <sup>ab</sup>	26.33 <sup>ab</sup>	39.00 <sup>ab</sup>	44.33 <sup>ij</sup>	41.67 <sup>hi</sup>
3o	80	8.33 <sup>f</sup>	12.33 <sup>hi</sup>	13.33 <sup>fg</sup>	16.00 <sup>d</sup>	103.00 <sup>b</sup>	96.33 <sup>b</sup>
	40	14.33 <sup>c</sup>	23.33 <sup>f</sup>	22.00 <sup>c</sup>	25.00 <sup>c</sup>	72.67 <sup>ef</sup>	70.00 <sup>f</sup>
	20	24.67 <sup>a</sup>	31.67 <sup>c</sup>	28.67 <sup>a</sup>	37.67 <sup>b</sup>	39.67 <sup>jk</sup>	36.33 <sup>ij</sup>
Carbofuran		4.00 <sup>h</sup>	7.33 <sup>k</sup>	9.00 <sup>h</sup>	12.67 <sup>d</sup>	132.33 <sup>a</sup>	115.00 <sup>a</sup>
Control		26.67 <sup>a</sup>	38.67 <sup>a</sup>	30.00 <sup>a</sup>	41.33 <sup>a</sup>	32.67 <sup>k</sup>	30.33 <sup>j</sup>
CD at 5%		2.25	3.47	3.76	4.26	11.02	8.27

No significant difference in mean with the same letters

shoot length, shoot weight, root length and root weight in comparison to control. The shoot length was found highest (38.50 cm) in plants treated with compound 3f followed by 3h > 3b > 3m > 3o @ 80 ppm at 60 DAI. Plants treated with 3f showed maximum (21.10 cm) root length followed by 3h > 3o > 3b = 3m @ 80 ppm at 60 DAI. The fresh shoot weight was found highest (10.05 g) in plants treated with 3f followed by 3h > 3o > 3b > 3m @ 80 ppm at 60 DAI. Plants treated with 3m showed highest (3.63 g) fresh root weight followed by 3f > 3b = 3o > 3h @ 80 ppm at 30 DAI.

The data (Table 2) showed that average no. of galls/plant at 30 and 60 DAI were significantly decreased as compared to control in soil drenching and root dipping method. In soil drenching method, it was found that average no. of galls/plant were minimum plants treated with 3f @ 80 ppm both (4.00) at 30 DAI and 60 DAI (8.00) followed by 3h < 3b < 3m < 3o. In root dipping method, it was found that average no. of galls/plant was minimum (9.67) in plants treated with 3f @ 80 ppm at 30 DAI. The order of average no. of galls/plant were found to be 3f < 3b = 3h < 3m = 3o @ 80 ppm at 30 DAI. And also, average no. of galls/plant was minimum (12.67) in plants treated with 3f @ 80 ppm at 60 DAI. The order of average no. of galls/plant were found to be 3f < 3h < 3b < 3m < 3o @ 80 ppm at 60 DAI. The data (Table 2) showed that yield (g) was significantly increased as compared to control in soil drenching and root dipping method. In soil drenching method, it was found that yield was maximum (124.33 g) in plants treated with 3f followed by 3h > 3o > 3b > 3m @ 80 ppm. In root dipping method, the yield was maximum (112.67 g) in plants treated with 3f followed by followed by 3h > 3m > 3o > 3b @ 80 ppm.

Based on findings, it can be concluded that all plant growth parameters (shoot length, shoot weight, root length, root weight, average no. of galls/plant and yield were positively influenced by these compounds. As the nematocidal activity of test compounds increased, plant growth parameters were also increased. The activity was highest in compounds 3f and 3h @ 80 ppm at 30 DAI and 60 DAI. The activity was higher in soil drenching method compared to root dipping method.

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