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RESEARCH ARTICLE

Effect of Methyl Salicylate on Rice Leaffolder *Cnaphalocrocis medinalis* (Guenee), Parasitoid *Xanthopimpla flavolineata* (Cameron) and Predator *Micraspis* sp.

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ABSTRACT

Cues associated with the host-plant complex, volatiles emitted by herbivore damaged plants and/or exogenous applications of elicitors decide on orientation behaviour of pests and beneficial insects. We conducted laboratory studies to know whether exogenous use of methyl salicylate (MeSA) influences on developmental stages and mortality of rice leaffolder, *Cnaphalocrocis medinalis* (Guenee), and biocontrol services of a ichneumonid parasitoid, *Xanthopimpla flavolineata* (Cameron) and a coccinellid beetle *Micraspis* sp. on *C. medinalis*. Results of green house studies revealed that MeSA at 100 mg L^{-1} significantly influenced the fecundity (38.4 eggs $female^{-1}$), egg hatchability (25.0%) and larval mortality of *C.medinalis* (75.0%) when compared to fecundity (75.0 eggs $female^{-1}$), hatchability (80.7%) and larval mortality (5.0%) due to buffer-treated control plants. MeSA at 100 mg L^{-1} also resulted in extended larval, pupal and adult duration of 7.11, 7.92 and 6.83 days, respectively as against the larval, pupal and adult duration of 6.08, 6.67 and 4.93 days, respectively in buffer treated control plants. MeSA at 100 mg L^{-1} also effected for lower pupal (28.5%) and adult (25.0%) emergence, than higher pupal (82.5%) and adult (85.0%) emergence in control plants. MeSA at 100 mg L^{-1} influenced for significant increase in the parasitization of *X. flavolineata* (70.0%) on *C. medinalis* pupae and predatory potential of *Micraspis* sp. (80.2%) on *C. medinalis* larvae. However, MeSA in any concentration was not lethal to both parasitoid and predator. There was significant difference in the attraction of *C. medinalis*, *X. flavolineata* and *Micraspis* sp. towards olfactometer arms due to MeSA treated and untreated leaves. The movement of *C. medinalis* was maximum towards leaves of control plants (5.5 adults with 28.0% attraction). This experiments revealed that MeSA treated rice leaves showed the less preference of *C. medinalis* adults (0.8 to 2.8 adults with 3.0 to 20.0% attraction). However, orientation behaviour of *X. flavolineata* (1.0 to 3.0 adults with 5.0 to 15.0% attraction) and *Micraspis* sp. (2.0 to 4.2 adults with 10.0 to 20.0% attraction) was higher towards leaves treated with MeSA concentrations.

Keywords: *Rice plants, Methyl Salicylate, Cnaphalocrocis medinalis, Developmental characteristics, Xanthopimpla flavolineata, Micraspis sp.*

Conservation biological control through ecological engineering pest management methods viz., crop habitat manipulation and use of herbivore-induced plant volatile (HIPV) elicitors is a valuable strategy to attract, conserve and enhance the biocontrol services of natural enemies (Gurr, 2009). Ecological engineering based chocolate box cropping methods have been developed for enhancing entomophages and natural pest suppression in cotton (Muthukrishnan *et. al.,* 2015), rice (Chandrasekhar *et. al.,* 2017), and blackgram (Lokesh *et. al.,* 2017). Since many plants show defense mechanisms through HIPVs, we wanted to study the uses of chemical elicitors in rice with a view to possibly alter volatile emissions to attract beneficial insects.

More specifically, upon herbivore attack, plants emit specific blends of volatiles that attract natural enemies of the herbivores. The importance of HIPVs as foraging cues has since been demonstrated in many systems, not only as attractants for natural enemies, (Arimura *et al.,* 2009, and Dicke *et al.,* 2010) but also, as repellents

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for herbivores (Snoeren *et al.,* 2010). These effects have prompted the notion that inducible volatile signals through HIPV elicitors can be enhanced in crop plants in order to improve the biological control of insect pests. These elicitors can activate various signalling pathways in the plant, causing an up-regulation of a large array of defense-related genes through cross-talk and resulting in accumulation or release of defense chemicals (Kessler and Baldwin, 2002). When plants are attacked by pest or pathogens, the defense mechanisms are triggered through signaling pathways, which comprise three plant signaling molecules: Salicylic Acid (SA), Jasmonic Acid (JA) and ethylene. The SA plays a substantial role in plant resistance signaling molecules (Wang *et al*., 2011).

Kalaivani *et al*. (2018) reported that the effect of MeSA at 100 mg L^{-1} exhibited greater mortality against rice leaffolder. Growth and development of *C. medinalis* was directly affected altering rates of ingestion, leaf area damage, stadia weights and development time, pupation, and successful pupation to adults were quantified. However, there is limited information on the effect of MeSA on the life history parameters of *C. medinalis* and its natural enemies. In this study, we addressed the aspects of MeSA treatment on the developmental characteristics of *C. medinalis* and parasitisation and predatory potential of *Xanthopimpla flavolineata* and *Micraspis* sp. along with mortality in green house and by olfactometer.

MATERIAL AND METHODS

Green house experiments were conducted at 27 ± 3°C, 65 ± 7% RH, and 12 L:12 D photoperiod in paddy breeding station (PBS) of Tamil Nadu Agricultural University (TNAU) and olfactometer studies were conducted at 28 ± 2°C, 60 ± 5% RH, and 12 L:12 D photoperiod in the laboratory of Chemical Ecology of the Department of Agricultural Entomology, TNAU, Coimbatore. 30 days old transplanted rice plants (cv. CO 51) maintained in pots along with quality soil and compost for the green house studies. Pot and soil were completely covered with aluminium foil to minimize possible effects of volatiles emitted by soil and compost. Mylar cages were used to cover the whole set up. Each experimental set up consisted of 21 potted plants representing seven treatments and three replications. Required fertilizers (25-0-20 N-P-K) and irrigation were given to the potted plants as and when needed.

Culturing and maintenance of insects

Different larval instars, pupae and adults of *C. medinalis* were collected from the wetland rice ecosystems of TNAU, Coimbatore and released in the 30 days old potted plants in the green house, and maintained continuously at 27 ± 3°C, 65 ± 7% RH, and 12 L:12 D photoperiod for the uninterrupted supply of eggs, different instars of larvae and adults for the laboratory experiments and olfactometer studies. Similarly, adults of *Micraspis* sp., and *X. flavolineata* were collected from the field and released in separate potted plants along with the early instar larvae and pupae of *C. medinalis* respectively and maintained continuously at 27 ± 3°C, 65 ± 7% RH, and 12 L:12 D photoperiod in the green house for the continuous supply to the laboratory experiments and olfactometer studies. Greenhouse studies were conducted to determine the developmental behaviour of *C. medinalis* and biocontrol services of *X. flavolineata* and *Micraspis* sp. at 27 ± 3°C, 65 ± 7% RH, and 12 L:12 D photoperiod. There were three replications in all the experiments. MeSA (Sigma – Aldrich, St. Louis, MO) was dissolved in sterile distilled water and dispersed in appropriate volumes to obtain 50, 75, 100, 125, 150, 200 mg L^{-1} concentrations. The potted plants arranged in three rows with 30 x 30 cm spacing on raised cement benches were sprayed with MeSA solutions of six concentrations as indicated above by using a regulator controlled hand sprayer. A set of buffer-treated (sterile distilled water) plants were maintained as control plants. All the potted plants were covered by using mylar cages.

To ensure oviposition, newly emerged *C. medinalis* adults, both males and females together were released at the rate of 10 $cage^{-1}$ and maintained till the death. Honey solution (20%) was given as adult food. Fecundity and per cent hatchability were recorded by number of eggs laid and the number of eggs hatched on leaves per day. First to fifth instar larvae taken from the mass culturing facility were released on the MeSA treated and control plants separately. The duration of each stadia like larval, pupal and adult and survival rate were recorded. Numbers of surviving larvae were counted and dead individuals were removed daily until pupation. Larval mortality was recorded by number of larvae died/ total number of released X 100. Thus percentage of mortality was calculated.

Similarly, two more sets of potted plants sprayed with six concentrations of MeSA and buffer-treated plants as indicated above were maintained separately. In one set, *C. medinalis* pupae from mass culturing facility at 20 $cage^{-1}$ were placed along with six numbers of *X. flavolineata* adults. Pupal parasitization was based on pupal mortality and adult emergence of *C. medinalis.* Likewise, adults of *Micraspis* sp. at 10 $cage^{-1}$ were released after the release of 30 second instar larvae of *C. medinalis* measured. Observation on predatory potential of *Micraspis* sp. was measured based on the number of larvae consumed per day.

Olfactometer study

Six-arm olfactometer was used to study the orientation behaviour of *C. medinalis, X. flavolineata* and *Micraspis* sp. Olfactometer experiments were conducted at 25 ± 2°C, 70 ± 10% RH, and L 12 : D12 photoperiod. The undamaged rice leaves treated with different doses of MeSA were placed in the arms of olfactometer and firmly closed with lid. The inlet of the olfactometer on the top center place was connected to an aquarium pump (220-240 volts AC) to release the pressure. In a six-arm olfactometer, control and six treatments were kept in two arms at the rate of two treatments and control at a single time. Pure air was passed from aquarium pump at the rate of 4 lit min^{-1} into the olfactometer. Twenty numbers of adults of *C. medinalis* were released to the olfactometer through a central hole which also served as odour exit hole. Observations were made on the number of adults settled on each arms at 5, 10, 15 and 20 min after release (MAR) for their host preference (Chandrasekar *et al.,* 2017). The experiment was replicated four times. Similar type of experiments were conducted with 20 adults of *X. flavolineata* and *Micraspis* sp.

The data from greenhouse and olfactometer meter experiments were scrutinized by CRBD analysis of variance (ANOVA) using AGRES after getting transformed into square root transformation (Gomez and Gomez, 2010). Critical difference values were calculated at five per cent probability level and treatments mean values were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Effect of MeSA on the developmental characteristics of Cnaphalocrocis medinalis

The data on the developmental characteristics of *C. medinalis* due to MeSA concentrations are given in Table 1. Our results indicated that fecundity (70.5 to 38.4 eggs $female^{-1}$), hatchability (65.2 to 25.0 %) of *C. medinalis* had negative correlation and larval mortality (42.8 to 75.0%) of *C. medinalis* with positive relationship with MeSA concentrations (50 to 250 mg L^{-1}). In all the three cases buffer treated control plants resulted in maximum fecundity (75.0 eggs $female^{-1}$) and hatchability (80.7%), and minimum mortality (5.0%) (Fig 1). MeSA at 100 mg L^{-1} influenced for higher mortality of *C. medinalis* larvae (75.0%), irrespective of the instars. The results are in corroboration with Kalaivani *et al.* (2018) who reported that the effect of MeSA at 100 mg L^{-1} exhibited greater mortality against the rice leaffolder. Decreased survival and increased mortality due to salicylate have been reported in several insect species including Helicoverpa zea (Li *et al*., 2002) and Culex quinquefasciatus (Mondal *et al*., 2014).

Table 1. Effect of MeSA on developmental characteristics of *Cnaphalocrocis medinalis* under greenhouse condition

MeSA concentrations	Fecundity (eggs/ female)	Hatchability (%)	Larval mortality (%)	Larval duration (Days)	Pupal development (%)	Pupal duration (Days)	Adult Emergence (%)	Adult longevity (Days)
50 mg L^{-1}	70.5^{d}	65.2^{d}	42.8^{e}	5.36^{e}	58.4^{f}	6.89^{bc}	55.2^{f}	5.33^{c}
75 mg L^{-1}	65.2^{c}	40.3^{c}	60.2^{d}	5.61^{d}	48.0^{e}	7.33^{b}	48.5^{e}	5.50^{c}
100 mg L^{-1}	38.4^{a}	25.0^{a}	75.0^{a}	7.11^{a}	28.5^{a}	7.92^{a}	25.0^{a}	6.83^{a}
125 mg L^{-1}	42.2^{b}	32.5^{b}	65.3^{c}	6.22^{c}	40.2^{c}	7.25^{b}	35.7^{c}	6.28^{b}
150 mg L^{-1}	40.5^{b}	30.2^{b}	70.5^{b}	6.69^{b}	35.6^{b}	7.31^{b}	30.5^{b}	6.47^{ab}
250 mg L^{-1}	41.6^{b}	38.5^{c}	62.2^{cd}	5.81^{d}	42.2^{c}	7.11^{bc}	38.2^{d}	6.33^{b}
Control	75.0^{e}	80.7^{g}	5.0^{f}	6.08^{c}	82.5^{g}	6.67^{c}	85.0^{g}	4.93^{d}
SED	0.08	0.90	1.01	0.02	0.52	0.04	0.84	0.04
CD (P = 0.05)	0.08	1.92	2.17	0.05	1.11	0.09	1.79	0.08

*Data are mean values of three replications

Figures were transformed by square root transformation; and arc sine transformation and the original values are given

In a columns means followed by same letter (s) are not significantly different (P=0.05) by DMRT

MeSA treatments resulted in prolonged larval (7.11 to 5.36 days), pupal (7.92 to 6.89 days) and adult duration (6.83 to 5.33), slowing the process of pupation (28.5 to 58.4%) and adult emergence (25.0 to 55.2%) of *C. medinalis*. This may be due to decreased feeding activity which leads to loss nutrition, and disturbances in the metabolic activities of larvae. Peng *et al*. (2004) revealed that MeSA played an essential role in triggering the defense mechanism in plants against herbivore attack. They observed that exogenous application of SA and MeSA to tomato seedlings induced the analogous genes that were triggered the plant after herbivore infestation and also increased the accumulation level of H_2O_2 against *Helicoverpa armigera* larvae feeding. It is possible to manipulate and enhance the existing indirect defense capabilities of plants by switching on relevant genes via the application of chemical elicitors so that their natural enemies are offered a reliable signal and are attracted only when they are likely to encounter prey or hosts on the crop (Pickett and Poppy, 2001).

Several of such elicitors have been shown to greatly invoke disease resistance by mediated effective defenses against pathogens and herbivores (Reyes *et al.,* 2010) and enhance the resistance in plants (Kalaivani *et al.,* 2018). One of the naturally existing phenolic compounds is salicylic acid (SA) which also acts as a growth regulator. Salicylic acid derivative of MeSA, methyl-2-hydroxybenzoate is an organic ester and natural product of many plant species.

Table 2. Effect of MeSA on mortality and biocontrol services of *Xanthopimpla flavolineata* and *Micraspis* sp. on *Cnaphalocrocis medinalis* under laboratory condition

MeSA concentrations	Parasitization of *Xanthopimpla flavolineata*		Predatory potential of *Micraspis* sp.	
	Parasitization (%)	Mortality (%)	Predatory potential (%)	Mortality (%)
50 mg L^{-1}	40.2^{c}	4.2	35.5^{f}	3.5
75 mg L^{-1}	42.3^{c}	4.5	42.8^{e}	3.0
100 mg L^{-1}	70.0^{a}	3.2	80.2^{a}	2.6
125 mg L^{-1}	65.4^{b}	3.8	70.5^{d}	3.4
150 mg L^{-1}	62.5^{b}	5.0	78.5^{b}	3.2
250 mg L^{-1}	64.6^{b}	4.0	76.8^{c}	3.4
Control	36.5^{d}	4.5	32.5^{g}	3.2
SED	0.82	NS	0.78	NS
CD (P = 0.05)	1.76		1.68	

*Data are mean values of three replications

Figures were transformed by arc sin transformation and the original values are given

In a columns means followed by same letter (s) are not significantly different (P=0.05) by DMRT

Effect of MeSA on mortality; and pupal parasitisation and predatory potential

Data on mortality and biocontrol services of *X. flavolineata* and *Micraspis* sp. due to MeSA concentrates are given in Table 2. Parasitization of *X. flavolineata* was significantly different in all the MeSA treatments compared to control. The maximum parasitization of *X. flavolineata* was registered due to MeSA at 100 mg L^{-1} (70.0 %) compared with natural condition, which contributed to 36.5 per cent. Mortality of *X. flavolineata* due to MeSA concentrations varied from 3.2 to 5.0 per cent and was non-significant. Regarding *Micraspis* sp., similar observation on the predatism on early instar larvae of *C. medinalis* was observed from 35.5 to 80.2 per cent due to exogenous application of MeSA on rice plants (Table 4). Predatory potential of *Micraspis* sp. was maximum in MeSA at 100 mg L^{-1} (80.2 %) with minimum predatism observed in control plants which contributed 32.5 per cent to MeSA concentrations. Mortality of *Micraspis* sp. due to MeSA concentrations varied from 2.6 to 3.5 per cent and was non-significant

Table 3. Behavioural response of adults of *Cnaphalocrocis medinalis*, *Xanthopimpla flavolineata* and *Micraspis* sp. towards MeSA concentrates by olfactometer

MeSA concentration	No. of *C. medinalis arm*$^{-1}$ at MAR*					No. of *X. flavolineata arm*$^{-1}$ at MAR*					No. of *Micraspissp. arm*$^{-1}$ at MAR*				
	5	10	15	Mean	% attraction	5	10	15	Mean	% attraction	5	10	15	Me an	% attraction
50 mg L^{-1}	2.0^{e}	2.9^{d}	3.5^{e}	2.8^{f}	20.0	0.3^{d}	1.2^{e}	1.5^{g}	1.0^{f}	5.0	1.4^{d}	2.1^{e}	2.5^{d}	2.0^{e}	10.0
75 mg L^{-1}	1.6^{d}	2.3^{c}	3.0^{d}	2.3^{e}	15.0	0.5^{d}	1.3^{e}	1.7^{f}	1.2^{e}	7.0	1.4^{d}	1.8^{f}	2.2^{e}	2.0^{e}	10.0
100 mg L^{-1}	0.4^{a}	0.8^{a}	1.2^{a}	0.8^{a}	4.0	1.7^{a}	3.2^{a}	4.2^{a}	3.0^{a}	15.0	1.5^{c}	4.5^{a}	5.0^{a}	4.2^{a}	20.0
125 mg L^{-1}	0.7^{c}	1.4^{b}	2.4^{c}	1.5^{d}	8.0	0.8^{d}	1.6^{d}	3.0^{c}	1.8^{d}	9.5	1.5^{c}	3.4^{d}	3.7^{c}	2.8^{d}	13.0
150 mg L^{-1}	0.5^{b}	0.9^{a}	1.6^{b}	1.2^{c}	5.0	1.5^{b}	2.8^{b}	3.5^{b}	2.6^{b}	12.0	2.5^{a}	3.9^{b}	4.3^{b}	3.6^{b}	17.5
250 mg L^{-1}	0.5^{b}	1.3^{b}	1.7^{c}	1.0^{b}	3.0	1.2^{c}	2.2^{c}	2.6^{d}	2.0^{c}	10.0	1.8^{b}	3.6^{c}	4.3^{b}	3.2^{c}	14.0
Control	3.5^{f}	6.2^{e}	6.7^{f}	5.5^{g}	28.0	0.2^{d}	1.0^{f}	1.8^{e}	1.0^{f}	5.0	1.2^{e}	1.6^{g}	2.0^{f}	1.6^{f}	7.0
SED	0.01	0.02	0.02	0.01		0.01	0.01	0.02	0.01		0.01	0.02	0.02	0.02	
CD (P = 0.05)	0.02	0.04	0.03	0.03		0.02	0.01	0.03	0.03		0.02	0.03	0.04	0.03	

* MAR: Minutes after release; Data are mean values of three replications

Figures were transformed by square root transformation and the original values are given

In a columns means followed by same letter (s) are not significantly different (P=0.05) by DMRT

Behavioural response of adults of Cnaphalocrocis medinalis, Xanthopimpla flavolineata and Micraspis sp. towards MeSA concentrates by olfactometer

Adults of *C. medinalis, X. flavolineata* and *Micraspis* sp. attracted towards MeSA- treated leaves at 5, 10, 15 and 20 min after release (MAR) placed in olfactometer are given in Table 3. There was significant difference in the attraction of *C. medinalis*, *X. flavolineata* and *Micraspis* sp. towards olfactometer arms due to MeSA treated and untreated leaves.

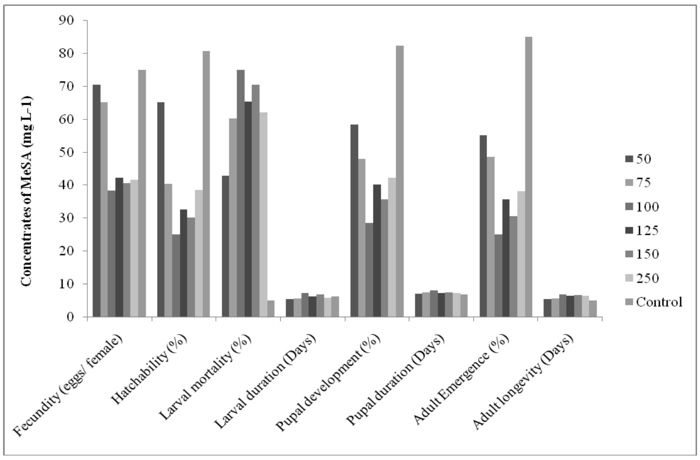

Figure 1. Effect of MeSA on developmental characteristics of *Cnaphalocrocis medinalis*

Orientation behaviour of C. medinalis

Out of 20 *C. medinalis* adults released, number of adults oriented towards MeSA treated and untreated leaves was minimum at 5 MAR (0.4 to 3.5) compared to 10 MAR (0.8 to 6.2) and 15 MAR (1.2 to 6.7). However, number of adults oriented towards arms due to MeSA treated leaves ranged from 0.8 to 2.8 than control plants recorded 5.5 adults. The order of preference of movement of *C. medinalis* was maximum towards buffer treated plants (5.5 adults with 28.0% attraction) followed by 50 mg L^{-1} (2.8 adults with 20.0 % attraction), 75 mg L^{-1} (2.3 adults with 15.0 % attraction), 125 mg L^{-1} (1.5 adults with 8.0 % attraction), 150 mg L^{-1} (1.2 adult with 5.0 % attraction), 250 mg L^{-1} (1.0 adult with 3.0 % attraction) and 100 mg L^{-1} (0.8 adults with 4.0 % attraction).

Orientation behaviour of X. flavolineata

Effect of MeSA on number of *X. flavolineata* adults oriented towards MeSA treated rice leaves and untreated leaves was minimum at 5 MAR (0.2 to 1.7) compared to 10 MAR (1.0 to 3.2) and 15 MAR (1.5 to 4.2) out of 20 adults released. However, the adults of *X. flavolineata* oriented towards arms due to MeSA treated leaves ranged from 1.0 to 3.0 than control plants, registered 1.0. The order of preference of movement of *X. flavolineata* was maximum towards 100 mg L^{-1} (3.0 adults with 15.0 % attraction), 150 mg L^{-1} (2.6 adults with 12.0 % attraction), 250 mg L^{-1} (2.0 adults with 10.0 % attraction), 125 mg L^{-1} (1.8 adults with 9.5 % attraction), 75 mg L^{-1} (1.2 adults with 7.0 % attraction), 50 mg L^{-1} and control plants (1.0 adult with 5.0 % attraction).

Orientation behaviour of Micraspis sp.

In case of *Micraspis* sp., there was a significant difference in the attraction of coccinellids by different doses of MeSA. Minimum orientation towards MeSA treated and untreated leaves at 5 MAR (1.4 to 2.5) compared to 10 MAR (1.6 to 4.5) and 15 MAR (2.0 to 5.0). But maximum coccinellid adults responded for the cues due to MeSA treated leaves when compared to *X. flavolineata* and *C. medinalis*. The number of adults of *Micraspis* sp. oriented towards arms due to MeSA treated leaves ranged from 2.0 to 4.2 than control plants (1.6). The order of preference of movement of *Micraspis* sp. was maximum towards 100 mg L^{-1} (4.2 adults with 20.0 % attraction), 150 mg L^{-1} (3.6 adults with 17.5 % attraction), 250 mg L^{-1} (3.2 adults with 14.0 % attraction), 125 mg L^{-1} (2.8 adults with 13.0 % attraction), 75 mg L^{-1} and 50 mg L^{-1} (2.0 adults with 10.0 % attraction) and control plants (1.6 adults with 7.0 % attraction).

Gui *et al.* (2004) reported that MeJA spray in tea could partially imitate the herbivore-induced defensive reactions against tea geometrid (TG) *Ectropis oblique* Prout, by braconid wasp, *Apanteles* sp., a parasitoid of TG in the glasshouse. Herbivory and application of JA to leaves caused the induction of volatile terpenoids that had specifically linked to the attraction of predator. This indicates that chemical elicitor is very useful as inducers of direct defenses in rice and indirect defenses mediated by predators and parasitoids.

We conclude that rice plants treated with MeSA resulted in decreased survival rate of *C. medinalis* and increased biocontrol services like pupal parastism and predatism of *X. flavolineata* and *Micraspis* sp., respectively. The results would certainly support efforts to improve rice yields and by providing more natural options in rice IPM by incorporating natural elicitors like MeSA.

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