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Identification of oviposition deterrents from pink bollworm, *Pectinophora gossypiella* (Saunders)

 V. Shah^{1*}, R. Pande¹, P. Verma², N. Gokte-Narkhedkar¹ and V.N. Waghmare³
¹Division of Crop Protection, ICAR-Central Institute for Cotton Research, Nagpur-440 010, India

²Division of Crop Production, ICAR-Central Institute for Cotton Research, Nagpur-440 010, India

³Division of Crop Improvement, ICAR-Central Institute for Cotton Research, Nagpur-440 010, India

 *Corresponding Author Email : vivek4256@gmail.com

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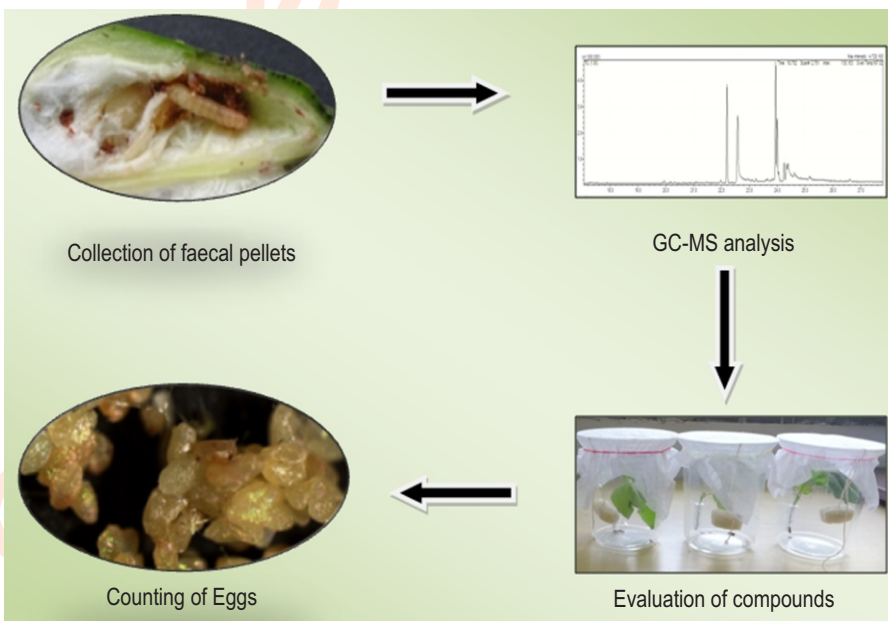
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Abstract

Aim : The present study was carried out to identify and explore novel areas of semiochemical based pest management like oviposition deterrents.

Methodology : The oviposition deterrents were identified from larval faecal pellets of pink bollworm using methanol as solvent and analysed in GC-MS. Three fatty acids were identified and evaluated for oviposition deterrent effect.

Results : In the present study, three major compounds namely; oleic, linoleic and palmitic acids were identified for the first time from larval faecal pellets of pink bollworm. Their oviposition deterrent effect was confirmed in bioassays carried out with different concentrations of identified compounds. The avoidance index (Ai) 0.78 ± 0.05 and per cent effective deterreny (PED) 87.42% was recorded in oleic acid at highest concentration followed by linoleic acid (Ai: 0.77 ± 0.03 ; PED: 86.61%) in reducing the egg laying by conspecific female. This clearly showed the role of these compounds as oviposition deterrent.

Interpretation : The compounds, oleic and linoleic acids evaluated in laboratory showed oviposition deterrent effect on female pink bollworm reducing egg laying considerably. However, further field studies need to be conducted to validate these observations.

Key words: Avoidance index, Effective deterreny, Faecal pellets, Fatty acids, Semiochemicals

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Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is the most destructive, cosmopolitan lepidopteran pest of cotton. The pest originates from South-Asian part of the world and was first described from India in 1842 from cotton and by mid-nineties subsequently spread across major cotton growing parts of the world (Ballou, 1920; Ingram, 1994; Byers and Naranjo, 2014). However, Indo-Pakistan origin of this pest was reconfirmed recently (Sridhar et al., 2017). Female moth lay eggs on squares, flowers or green bolls. The destructive larvae of pink bollworm usually feeds on flower buds, bolls and seeds therein, which results in malformation, rotting, premature or partial boll opening, reduction in fibre length and overall deterioration in the quality of cotton crop due to staining of the lint. The larval stage is usually hidden within the cotton fruiting bodies making them unreachable by insecticidal sprays owing to which its management is a difficult task for cotton growers. This marks the importance of this pest in cotton production system.

Pink bollworm remains to be the pest of global concern with its most destructive nature of feeding habit known to cause economic loss in seed cotton yield to the extent of 2.8 to 61.9 per cent, reduction in oil content to the tune of 2.1 to 47.1 per cent and 10.7 to 59.2 per cent poor opening of bolls (Shrinivas et al., 2019). After initial introduction of *Bt*-cotton as Bollgard I in 2002 and subsequently Bollgard II in 2006, the cotton crop could with stand the bollworms till 2010. The first incidence of pink bollworm on Bollgard-II was reported from Amreli in Gujarat which showed mean survival of 72% at diagnostic concentration *Cry1Ac* (Dhurua and Gujar, 2011). Subsequent research studies from Monsanto in 2010 confirmed the *Cry1Ac* resistance reporting unusual survival of pink bollworm on *Bt* cotton during 2009 in four districts of Gujarat viz., Amreli, Bhavnagar, Junagarh and Rajkot. Diet incorporation bioassays to evaluate resistance levels of *Cry1Ac* at diagnostic concentration ($10 \mu\text{g ml}^{-1}$) in two populations collected from *Bt* cotton (Anand, Gujarat) and non-*Bt* cotton (Akola, Maharashtra) fields during 2010-11 were done. The population collected on *Bt* cotton showed survival of 65% whereas complete mortality was observed in non-*Bt* field collected population (Fabrick et al., 2014). Recently, pink bollworm resistance in *Bt* cotton to both *Cry1Ac* and *Cry2Ab* has been reported from India indicating future threat to cotton cultivation (Naik et al., 2018). To address this dual problem of resistance to *Bt* toxins and ineffectiveness of insecticides to reach target insect due to concealed feeding habit of pink bollworm (Lykouressis et al., 2005), there is a need to develop an alternative control strategies for its management.

One of the best options is application of info-chemicals that disrupt feeding, mating and oviposition behaviour in insect. Oviposition deterrent from larval faecal pellets have been documented to reduce oviposition of conspecific females in an array of lepidopteran insects studied (Renwick and Radke, 1980; Ditttrick et al., 1983; Williams et al., 1986; Klein et al., 1990; Anderson and Lofqvist, 1996; Rhainds et al., 1996). Similarly,

oviposition deterrents have been identified and validated in many coleopteran species as well (Anbutsu and Togashi, 2002; Anderson, 2002). The specificity of deterrents have been proved with a single blend of compounds identified from *Ostrinia zealis* that remains effective for other species within the same genus (Li and Ishikawa, 2004). Howlader and Ambadkar (1995) reported 82% oviposition deterrence in whole body wash extract of tobacco beetle, *Lasioderma serricorne* against conspecific female. The studies on per cent effective deterrence (PED) or per cent oviposition deterrence (OD%) in blow fly species, *Lucilia sericata* using essential oils had shown the deterrence more than 80%, after 24 h of incubation (Bedini et al., 2019). Studies on the chemical identification and detection of larval faecal pellet-originated oviposition deterrents from pink bollworm have not been attempted so far, which may be a great alternative as ethological pest management for these insects. In the present investigation, an attempt was made to explore the role of these chemicals in formulating oviposition deterrent based management strategy.

Materials and Methods

Insect culture collection and maintenance: Pink bollworm larvae collected from Nagpur, Maharashtra, India from cotton (*Gossypium hirsutum* L.) variety Suraj during 2017-18 were reared on natural food, i.e., on cotton (*G. hirsutum*) bolls and on artificial diet under controlled conditions ($65 \pm 5\%$ relative humidity (RH); 14L:10D photoperiod $27 \pm 1^\circ\text{C}$ temperature) in an insectary. Faecal pellet was collected from larvae reared on cotton bolls for GC-MS analysis. Whereas for bioassays on adult response towards oviposition deterrence as moth number is required in huge quantity population was reared on artificial diet. The male and female sexes were separated at larval stage itself as male larvae have two dots (testes) on dorsum of 5th abdominal segment, which is otherwise absent in female. The pupae were kept separate up to adult emergence. Total 30 male and female moths were chosen after eclosion and transferred to a plastic container, covered with muslin cloth. Cotton twig containing squares was provided as an oviposition substrate to moths. Moths were supplied with cotton plug dipped in 10% honey solution as food. Moths were allowed to pair and lay eggs on cotton twigs. Base of cotton twigs were dipped in eppendorf tubes provided with water and covered with parafilm to keep twigs fresh for long time.

Collection of faecal pellet : Fresh larval faecal pellet was collected in methanol (1 mg faecal pellet/10 μl) from pink bollworm, reared on cotton bolls and were incubated overnight at 4°C . The supernatant was subjected to GC-MS analysis for identification of compounds.

Gas chromatography-mass spectrometry (GC-MS) analysis: Compounds extracted in HPLC grade methanol (Himedia®) from larval faecal pellet of pink bollworm were subjected to GC-MS (Schimadzu QP-2020 system) analysis. Capillary non-polar phenylene dimethyl polysiloxane capillary column (Rxi-5 Sil MS) with dimension 0.25 mm x 30 m x 0.25 μm was used for

separation and identification of compounds was done using NIST mass spectral library. Helium (99.99% purity, LabPulse India Ltd) was used as carrier gas. The split less mode of injection was used with inlet temperature of 280°C. The oven temperature programmed maintained at initial temperature of 40°C with 3 min hold and a ramp of 10°C/min till 250°C and held for 25 min with column (Rxi-5 Sil MS) flow of 1.4 ml/min with linear velocity of 38.6 cm/sec and pressure of 60 kPa. Mass spectral detector was maintained at a temperature of 200°C with the interface temperature of 260°C. Sample was injected into the column in 1 µl aliquots. Finally, for identification and quantification of compounds, data was evaluated by TIC (Total ion chromatogram). The mass spectra generated using MS was compared with the stored data base of NIST mass spectral library (NIST 2014 version).

Bioassay: Bioassays were carried out under the following environmental conditions: 65 ± 5% relative humidity; 14L:10D photoperiod, 27 ± 1°C temperature in the insectary as outlined for larval rearing. Before the test, about five pairs of newly emerged moths were paired in plastic container (13.5 cm H and 11.5 cm D) during scotophase for arbitrary mating provided with cotton plug dipped in 10% honey solution as food source. Mating was allowed for 48 hr. Cotton twig from cotton variety Suraj containing square was provided as an oviposition substrate. Twigs were treated with desired concentration (0.2, 0.4, 0.6, 0.8 and 1.0%) of identified compounds. Twigs treated with water and methanol (diluent) was used as control. After evaporation of the solvent, treated twigs were provided to mated females. The experiments were terminated on 10th day after the twig was provided. In all tests, the numbers of eggs for control (C) and treatments (T) were counted. The Avoidance index (A) (Renwick and Radke, 1980) and per cent effective deterrency (PED) (Rajkumar and Jebanesan, 2009) was calculated by taking into consideration reduction in number of eggs laid over control.

Statistical analyses: Statistical software SPSS Version 16.0 for windows was used to calculate mean and standard error. For comparison of mean values, Tukey's HSD (honest significant difference) test at P=0.05 level of significance was used (SPSS, 2007).

Results and Discussion

(Data not provided here). The active deterrence crude extract was subjected for component identification using GC-MS. The previous studies conducted on identification of oviposition deterrent compounds from different substrates (egg, faecal pellet, tarsi, abdomen, scales, anal tuft) across diverse insect groups have proved the oviposition deterrent activity using crude extract, artificial compounds either solely or in combination. Available reports on identification of oviposition deterrents have clearly shown that fatty acids and their methyl esters are the main compounds having oviposition deterrent effect as found in the present study. Many studies have been conducted on identification of compounds solely from eggs. Thiery and Le

Quere (1991) found the blend of oleic and palmitic acid along with their methyl esters as major compounds from eggs of *Ostrinia nubilalis*. According to Thiery et al. (1992a and 1992b), combination of fatty acids and methyl esters, namely hexadecanoic, 9-hexadecenoic, (Z)-9-octadecenoic, 9,12-octadecadienoic and octadecanoic acid were the active compounds present in the eggs of *Lobesia botrana* and *O. nubilalis*. Blend of myristic, palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid was reported from egg extract of *L. botrana* (Gabel and Thiery, 1996). Fatty acids of varying chain length ranging from C14 to C20 were found in egg mass of *O. scapularis* with C16:0 being most abundant followed by palmitoleic acid (C16:1) and oleic acid (C18:1) (Li and Ishikawa, 2004; 2005). Oleic acid (C18:1) and palmitoleic acid (C16:1) and their methyl esters were the active compounds present in the egg masses of cabbage seed weevil, *Ceutorhynchus assimilis* (Mudd et al., 1997). From eggs of *Cydia pomonella*, myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and alpha-linolenic acid (C18:3) were identified as oviposition deterrents (Thiery et al., 1995).

GC-MS analysis of larval faecal pellet samples collected in methanol revealed the presence of three fatty acids and four methyl esterified forms. The compounds were further quantified using fatty acid standards from Sigma Aldrich®. Upon quantification, oleic acid (9-octadecenoic acid) (323.53±1.55 ppm), linoleic acid (9,12-octadecadienoic acid) (155.94±6.06 ppm) and palmitic acid (hexadecanoic acid) (113.73±2.39 ppm) were identified as major compounds. The quantity of methyl ester derivatives was negligible (less than 15 ppm) which would have been derived due to use of methanol as solvent. Study conducted on identification of compounds in larval faecal pellets of four lepidopteran species (*Ostrinia furnacalis*, *O. scapularis*, *O. zealis*, and *O. latipennis*) reared on artificial diet have also confirmed the blend of five fatty acids, palmitic, stearic, oleic, linoleic and linolenic acids (Li and Ishikawa, 2004).

Similar blend of fatty acids, palmitic and oleic acid in the ratio of 1:1 has been identified from the egg and/or faecal pellet extracts of *Helicoverpa armigera* that produced oviposition deterrent effect (Li et al., 2001). Identification of oviposition deterrents having blend of fatty acids and their corresponding methyl esters have been documented in many species of lepidoptera as per the literature reports. Blend of fatty acids proved to be oviposition deterrent identified in larval frass extract of *H. armigera* contained myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1) along with their methyl esters were identified (Li et al., 2001; Xu et al. 2006). The main components in larval frass of *O. zealis* were five free aliphatic fatty acids, palmitic, stearic, oleic, linoleic, and linolenic acids (Li and Ishikawa, 2004). Study conducted on identification of compounds in larval frass of four lepidopteran species (*O. furnacalis*, *O. scapularis*, *O. zealis*, and *O. latipennis*) reared on artificial diet have also shown blend of five fatty acids, palmitic, stearic, oleic, linoleic, and linolenic acids (Li and Ishikawa, 2004; 2005). Our results were in concurrence with these reports, where

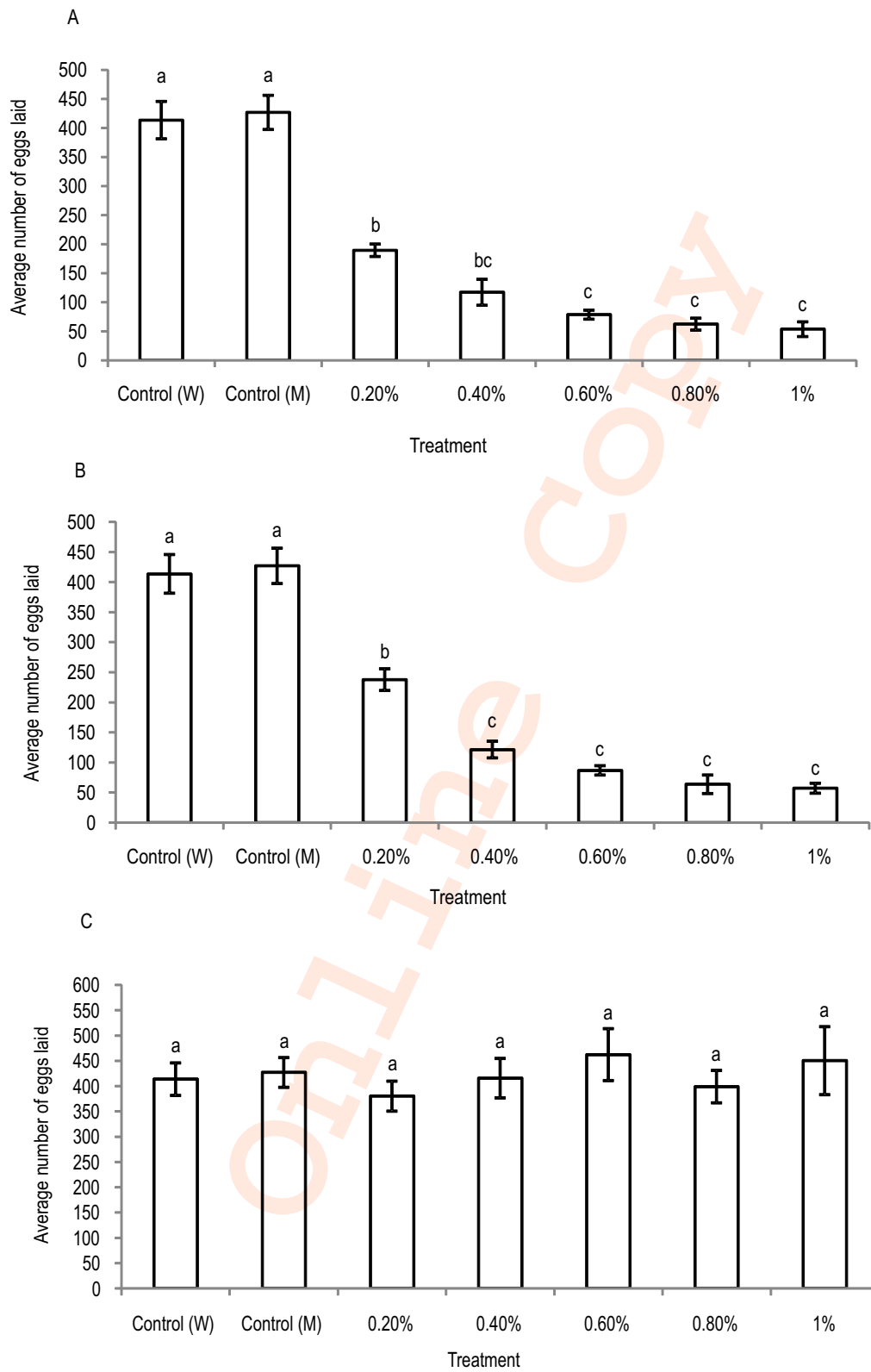


Fig. 1: Average number of eggs laid in (A) *Oleic*; (B) *Linoleic* and (C) *Palmitic* acid treatment. Values are mean of replicates \pm S.E. Bars followed by same letters are not significant at $P=0.05$ Tukey's HSD (honest significant difference).

we found three fatty acids palmitic acid (hexadecanoic acid, C16:0), linoleic acid (9, 12-octadecadienoic acid, C18:2) and oleic acid (9-octadecenoic acid, C18:1) along with methyl esters of four fatty acid palmitic acid (hexadecanoic acid, C16:0), linoleic acid (9, 12-octadecadienoic acid, C18:2), oleic acid (9-octadecenoic acid, C18:1) and stearic acid (octadecanoic acid, C18:0). In the present investigation, blend of three fatty acids viz., oleic, linoleic and palmitic acid along with their methyl esters were identified. As the concentrations of methyl esters were almost negligible, only fatty acids alone were evaluated for oviposition deterrent effect. This is in agreement with the study conducted in egg extracts of *Ostrinia nubilalis* (Thiery and Le Quere, 1991) where they identified the blend of oleic and palmitic acid along with negligible amount of their methyl esters. Bioassay was performed using five different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) of each compound following the standard protocol. A significant difference was observed with increasing concentration of individual compound in case of oleic and linoleic acid. The test of significance did not hold good for palmitic acid where all concentrations were on par with control. The mean number of eggs laid in each set of experiments with three different fatty acids is depicted in Fig. 1. The mean number of eggs laid in water (413.57 ± 32.15) and methanol (426.86 ± 29.38) control were significantly different from the lowest concentration (0.2%) of oleic acid (189.57 ± 10.73) and linoleic acid (237.71 ± 18.03). Palmitic acid did not show any significant difference across all the concentrations (Fig. 1).

In order to check the efficacy of compound and preference of conspecific female for egg laying on treated surface A_i and PED were calculated. The study clearly showed that oleic acid had A_i ranging from (0.37 to 0.76) compared to water treated control. The A_i increased with increase in concentration of oleic acid but not significant from the concentration of 0.6% conc. (0.68) and above. The PED ranged from of 54.16% to 87.01% for oleic acid compared to water treated twig similarly. A_i (0.38-0.78) and PED (55.59%-87.42%) were found for methanol treated twigs. No significant difference was observed between methanol and water treated twigs indicating PED to be 0.00. Comparable results were obtained using increasing concentrations of linoleic acid as A_i (0.27 ± 0.04 - 0.75 ± 0.04) and PED (42.52-86.18%) when compared with water treated twig as control. In case of methanol treated twig as control, the A_i and PED values ranged from 0.28-0.77 and 44.31-86.61%, respectively.

Similar to the results of oleic acid here as well no significant increase in effect was observed with increase in concentration above 0.6%. Though, palmitic acid was one of the major identified compounds, however, no significant effect on egg laying was recorded in terms of either A_i (-0.05-0.04 when compared with water control; -0.03-0.06 when compared with methanol control) or PED (-11.74-8.12% when compared with water control; -8.27-10.98 when compared with methanol control). The larval faecal pellet extract and their identified components have shown significant oviposition-deterrent effects in this experiment. The avoidance index of 0.37-0.76, 0.27-0.75

(water control) and 0.38-0.78, 0.28-0.77 (methanol control) were observed in oleic acid, and linoleic acid respectively. The avoidance index value of *Agrotis segetum* (Anderson and Lofqvist, 1996) and *O. nubilalis* (Dittrick et al., 1983) which was as high as 0.8, comparable values were also obtained in the present study. However, Li and Ishikawa (2004) reported lower values of avoidance index (0.28 - 0.55) in four *Ostrinia* species.

Similarly, per cent effective deterrence in present experiment, 54.16 - 87.01% (water control) and 55.59 - 87.42% (methanol control) for oleic acid and 42.52 - 86.18% (water control) and 44.31 - 86.61 (methanol control) for linoleic acid was supported by the work of Howlader and Ambadkar (1995), who studied the oviposition deterrence in whole body wash extract of tobacco beetle, *Lasioderma serricornis* in hexane, and found 82% deterrence against conspecific female. Studies conducted in blow fly species, *Lucilia sericata* using essential oils showed deterrence greater than 80%, after 24 hr of incubation (Bedini et al., 2019). Various studies have been carried out in many lepidopteran species where significant reduction in egg laying was observed (Thiery and Le Quere, 1991; Anderson and Lofqvist, 1996; Li and Ishikawa, 2004)

However, the results in most of the studies have been reported in absolute number of eggs reduced after treatment rather than per cent effective deterrence.

In the process of oviposition, female insect releases intentionally or perchance blend of fatty acids on host plant surface as its 'footprints' (Li et al., 2001) to minimize intraspecific competition for resources. A profile of seven compounds was detected in faecal pellets of pink bollworm with three as major compounds, in which two compounds were found promising, i.e., oleic and linoleic acid. Given the indiscriminate use of pesticides and subsequent resistance development of target pest, promising deterrent compounds identified in the present study would serve a better option for the management. However, these compounds need to be further evaluated in combinations for effective and eco-friendly pest management option under field conditions.

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