Larvicidal and Structure–Activity Studies of Natural Phenylpropanoids and Their Semisynthetic Derivatives against the Tobacco Armyworm *Spodoptera litura* (FAB.) (Lepidoptera: Noctuidae)[1])

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The larvicidal activity of 18 phenylpropanoids, **1**–**18**, including phenylpropenoate, phenylpropenal, phenylpropene, and their semisynthetic analogues, were evaluated against the tobacco armyworm, *Spodoptera litura* (FAB.), to identify promising structures with insecticidal activity. Amongst various phenylpropanoids, isosafrole, a phenylpropene, showed the best activity, with an LC_{50} value of 0.6 μg/leaf cm^2, followed by its hydrogenated derivative dihydrosafrole (LC_{50} = 2.7 μg/leaf cm^2). The overall larvicidal activity of various phenylpropene derivatives was observed in the following order: isosafrole (**6**) > dihydrosafrole (**16**) > safrole (**12**) > anethole (**4**) > methyl eugenol (**11**) > eugenol (**13**) > *β*-asarone (**8**) > dihydroasarone (**18**) > dihydroanethole (**15**). Dihydrosafrole might be a promising compound, although presenting a lower larvicidal activity than isosafrole, because of its better stability and resistance to oxidative degradation (due to the removal of the extremely reactive olefinic bond) in comparison to isosafrole. Such structure–activity relationship studies promote the identification of lead structures from natural sources for the development of larvicidal products against *S. litura* and related insect pests.

Introduction. – One of the important concerns in pest management strategies is to reduce the use of conventional chemical pesticides, due to their inherent potency to induce resistance among the target insect species [1][2] and their potential harmful effects on human health, environment, and non-target organisms [3]. In this context, there have been increasing efforts in exploring the possibilities of developing plant-based insecticides [4–6] or their semisynthetic derivatives [7]. Research approaches in this direction include: *i*) screening programs to explore new potential flora, *ii*) exploiting the published information on the insecticidal potential of plants for developing viable formulations of active principles or blends from the abundantly available source, and *iii*) exploiting the lead compounds from promising chemical families, either to use them for developing insecticide formulations, or as a prelude for structure–activity relationship (SAR) studies to obtain synthetic or semisynthetic leads with superior performance. The knowledge on lead compounds is generally gained by bioassay-guided fractionations of promising plant sources, followed by the identification of marker compounds responsible for the desired activity.

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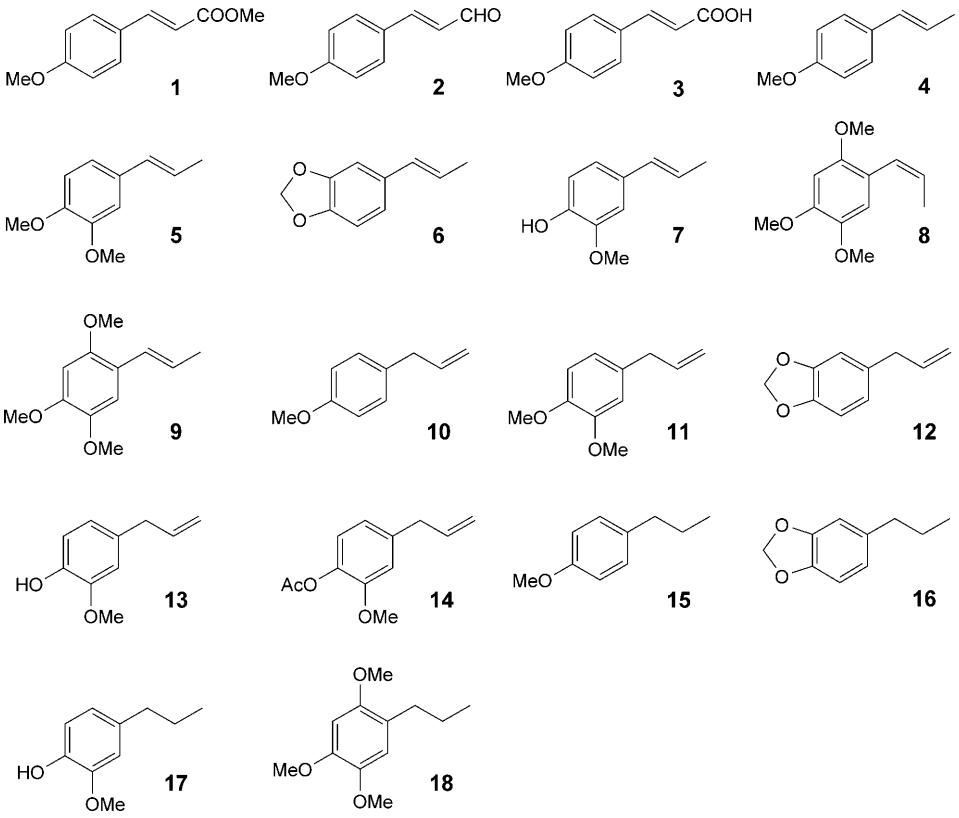
Our effort in this direction is primarily focused on exploring the potential of phenylpropanoids as insecticidal agents. Phenylpropanoids (C_6–C_3 unit), a major group of naturally occurring phenolic compounds in plants, have attracted our interest to evaluate their insecticidal properties, because of their involvement in plant defense against pests [8–10]. The main objective of the present study is to identify the promising insecticidal structures of natural phenylpropanoids, followed by understanding the SAR by modifying the structures to obtain leads with superior performance. The insecticidal potential of abundantly available natural phenylpropanoids was evaluated against *Spodoptera litura*, one of the most widespread and economically important crop pests [11].



Results and Discussion. – *Preliminary Screening to Identify a Lead Structure.* Phenylpropanoids were tested against larvae of a lepidopteron insect, *Spodoptera litura*. Host plant (castor oil plant, *Ricinus communis*) leaves were treated with a formulation containing the desired compounds at different dosages and allowed to feed by the starved larvae. The effect of these compounds on the larval mortality was observed and compared with the controls, as explained in details in the *Exper. Part*. The preliminary screening for insecticidal activity of phenylpropanoids with four different functional groups, *viz.*, an ester (methyl 4-methoxycinnamate; **1**), an aldehyde (4-

methoxycinnamaldeyde; **2**), an acid (4-methoxycinnamic acid; **3**), and an olefin (4-methoxyphenylpropene or anethole; **4**), against the larvae was carried out at 15 μg/leaf cm^2 (*Table 1*). Compound **4**, a phenylpropene with a C=C bond adjacent to C(1), showed a promising toxic activity against the larvae. The other three compounds included in the preliminary screening did not show appreciable activities. Hence, compound **4** was selected as starting point for further SAR investigations, because of its larvicidal activity and the abounding availability of **4** and its related phenylpropenes in nature [8].

Table 1. *Larvicidal Activity of Phenylpropanoids against* Spodoptera litura *at 15 μg/leaf* cm^2

Compound	Mortality [%][a])		Compound	Mortality [%][a])	
	24 h	48 h		24 h	48 h
1	–[b])	–	**3**	–	–
2	–	–	**4**	86.6±6.0	100±0.0

[a]) Mortality corrected for the mortality of the untreated control insects using *Abbott*'s formula; values are means and standard-deviations of three replicates. [b]) Activity <10%.

Semisynthetic Derivatives of Phenylpropenes and SAR. A series of compounds related to 4-methoxyphenylpropene (**4**), obtained either from natural sources or by semisynthetic routes (**5**–**18**), were evaluated for their larvicidal activities, to understand the effect of various substitution patterns on their performance. The insecticidal potential of these compounds is compiled in *Table 2*. Some of the compounds, *i.e.*, anethole (**4**), isosafrole (**6**), β-asarone (**8**), methyl eugenol (**11**), safrole (**12**), eugenol (**13**), and dihydrosafrole (**16**) induced appreciable larval mortality (≥60%) after 48 h of exposure time. Increasing the exposure time had a significant (at the 5% level) effect on the larval mortality for compounds **4**, **8**, **11**, and **13**, while no significant difference in activity was observed for compounds **6**, **12**, and **16**. Based on the larvicidal potential of the compounds (*Table 2*), promising lead structures were identified and their toxicities were quantified by means of dose–response lines.

Table 2. *Larvicidal Activity of Phenylpropenes against* Spodoptera litura *at 15 μg/leaf* cm^2

Compound	Mortality [%][a])		Compound	Mortality [%][a])	
	24 h	48 h		24 h	48 h
5	–[b])	–	**12**	96.0±6.0	100.0±0.0
6	100.0±0.0	100.0±0.0	**13**	26.0±6.0	66.0±0.0
7	–	–	**14**	–	16.0±15.3
8	16.0±11.5	60.0±0.0	**15**	53.0±6.0	53.0±6.0
9	–	–	**16**	100.0±0.0	100.0±0.0
10	–	–	**17**	10.0±0.0	23.0±6.0
11	36.0±15.3	76.6±11.5	**18**	–	56.0±6.0

[a]) Mortality corrected for the mortality of the untreated control insects using *Abbott*'s formula; values are means and standard-deviations of three replicates. [b]) Activity <10%.

LC_{50} Values and other statistical parameters, generated by linear regression analysis, are listed in *Table 3*. Isosafrole (**6**) was the most active compound, with an LC_{50} value of 0.6 μg/leaf cm^2 after 48 h of exposure, whereas dihydroanethole (**15**) was the least toxic among the compounds tested in the series. Overall, the activity of the compounds after 48 h of exposure decreased in the order: isosafrole (**6**) > dihydrosafrole (**16**) > safrole (**12**) > *trans*-anethole (**4**) > methyl eugenol (**11**) > eugenol (**13**) > *β*-asarone (**8**) > dihydroasarone (**18**) > dihydroanethole (**15**). It can also be noted that, in general, the LC_{50} value of compounds was negatively correlated with the exposure time. Interestingly, there was not much difference in the activity between 24 and 48 h of exposure for compounds **4**, **6**, **12**, **15**, and **16**. It was also observed that compounds **8**, **11**, and **13** showed delayed effects with very low mortalities at 24 h contact time in the tested concentration range. Hence, LC_{50} values (12.7, 9.9, and 11.4 μg/leaf cm^2, resp.) for these compounds could be calculated only after 48 h of exposure. The activity of **6**, the most active phenylpropene, was comparable with that of the biological (*NeemAzal*) and the chemical (methyl parathion) reference insecticides at 48 h contact time (*Table 3*).

Table 3. *Larvicidal Activity* (LC_{50} values and regression parameters of probit analysis) *of the Promising Compounds against* Spodoptera litura

Compound	24 h			48 h		
	LC_{50} [μg/leaf cm^2]	χ^2	Slope ± SE	LC_{50} [μg/leaf cm^2]	χ^2	Slope ± SE
4	9.4 (7.5–11.3)	4.1	1.4 ± 0.2	8.0 (6.9–9.2)	5.2	2.4 ± 0.4
6	2.1 (1.5–2.7)	0.8	1.4 ± 0.2	0.6 (0.2–1.0)	0.3	1.0 ± 0.3
8	–	–	–	12.7 (10.4–15.5)	1.1	1.3 ± 0.2
11	–	–	–	9.9 (8.1–11.9)	0.5	1.4 ± 0.2
12	8.5 (7.4–9.5)	0.2	3.3 ± 0.5	7.6 (6.7–8.6)	0.5	3.7 ± 0.6
13		–	–	11.4 (9.3–14.0)	0.8	1.3 ± 0.2
15	14.5 (12.0–17.9)	0.0	1.4 ± 0.2	14.2 (11.7–17.6)	0.1	1.3 ± 0.2
16	3.2 (2.3–4.1)	4.1	1.3 ± 0.2	2.7 (1.9–3.5)	4.8	1.3 ± 0.1
18	–	–	–	13.9 (11.5–17.2)	0.2	1.3 ± 0.2
NeemAzal	7.0 (5.2–15.0)	1.7	1.0 ± 0.3	2.6 (1.6–4.8)	0.6	0.4 ± 0.1
Methyl parathion	0.6 (0.5–0.6)	2.8	1.1 ± 0.5	0.3 (0.3–0.3)	4.3	1.2 ± 1.3

[a]) LC_{50} value was not determined due to the low mortality in the tested concentration range.

Most of the studies on phenylpropanoids pertaining to insecticidal activities [12][13] are limited to and aiming at evaluating the activities of the individual compounds against different target species of interest. In the present study, our effort was to identify and select the most promising structure among the basic phenylpropanoids, based on preliminary screening results and source availability of the compounds, followed by understanding the SAR of the promising compounds and their related derivatives to obtain leads with enhanced activity. Though many of these phenylpropanoids occur in plants, a lot of them are not available in sufficient quantity and purity for their bioactivity evaluation [14]. It is also worth mentioning that some of the dihydrophenylpropanoids have not been previously tested against *S. litura*.

In the first part of the study, the larvicidal activity of various phenylpropanoids containing different functional groups was evaluated and compared (*Table 1*). None of

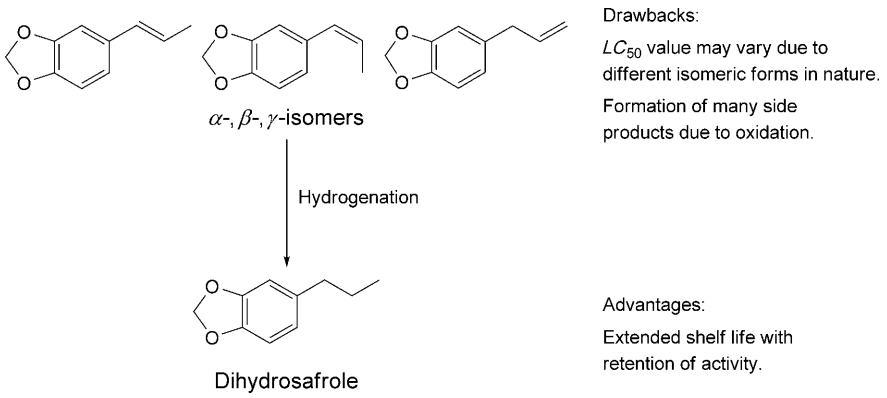
the compounds having a functional group, such as an ester, aldehyde, or acid group, attached at C(3) of the alkyl chain showed encouraging activity. However, the phenylpropene **4**, with a C=C bond adjacent to C(1) of the alkyl chain, demonstrated promising activity. Thus, it was observed that functional groups attached to the alkyl side chain played a major role in contributing to the activity, which is in agreement with earlier studies [8][9]. Considering the availability and economics, **4** and the related phenlypropenes **5**–**18** were further investigated.

Antifeedant Effect. We also observed the antifeedant activities for compounds **5**–**18**, and the following antifeedant effects were found: >90% for anethole (**4**), isosafrole (**6**), safrole (**12**), methyleugenol (**11**), β-asarone (**8**), and dihydrosafrole (**16**), between 60 and 80% for eugenol (**13**) and dihydroeugenol (**17**), and between 40 and 60% for dihydroasarone (**18**), dihydroanethole (**15**), and isoeugenol (**7**). However, possible physiological effects of these compounds on the larvae and thereby on their subsequent feeding behavior could have affected the measurement and judgment of the antifeedant activities.

Larvicidal Effect. Phenylpropenes occur in three isomeric forms, α, β, and γ, in nature. In α- or β-isomers, the C=C bond is adjacent to C(1), where as in γ-isomers, the C=C bond is adjacent to C(2) of the alkyl side chain (*Scheme*). It is known that the activity of phenylpropenes is greatly influenced by the variations in the isomeric forms and/or functionalities attached to either the aryl ring or the alkyl side chain of C_6–C_3 systems. In the present study, during the initial screening of phenylpropenes with different functionalities, anethole (**4**), isosafrole (**6**), β-asarone (**8**), methyl eugenol (**11**), safrole (**12**), eugenol (**13**), dihydroanethole (**15**), and dihydrosafrole (**16**) showed promising activity (*Table 2*) and were subjected to the dose–response bioassay to quantify their toxicity (*Table 3*). Compound **4**, with one MeO group at C(4) of the aromatic ring, in *trans*-form, was active (LC_{50} value of 8.0 μg/leaf cm^2), whereas its γ-counterpart, *i.e.*, methyl chavicol (**10**), did not show activity. In contrast, isoeugenol (**7**), with OH and MeO groups at C(4) and C(3) of the aromatic ring, respectively, was inactive (0% mortality), whereas the γ-isomer with the same substitution pattern (**13**) showed good insecticidal activity with an LC_{50} value of 11.4 μg/leaf cm^2 after 48 h of exposure. Reports on the enhanced activity of phenylpropenes by non-polar substitutions at the aromatic ring, (*e.g.*, MeO *vs.* OH groups) in general [15], and sensitivity of pests to MeO substituted compounds in particular, prompted us to screen methylisoeugenol (**5**) and methyleugenol (**11**) with two MeO groups at C(3) and C(4). It was observed that only the γ-isomer **11** was active (*Table 2*). On altering the polarity of **11** by replacing the MeO group at C(4) by an AcO group, *i.e.*, compound **14**, caused a considerable decrease in activity, which suggests the dominant role of polar groups in influencing the activity. *Harmatha* and *Nawrot* [15] have also reported similar observations of reduced activity on substitution of polar groups in phenylpropanoids against some stored grain pests. In another report on acaricidal activity of phenylpropenes [16], it was shown that bioefficacy increased linearly with molecular lipophilicity. The activity of compounds **4** and **11** turned our attention to compound **8**, another closely related phenylpropene. Comparison of the activity of **8** with that of the two related structures **4** and **5** (*Table 3*), with the only difference being the MeO group substitution on the aromatic ring, shows that the effect of multiple apolar substituents is additive and the presence of the C=C bond at C(2) of the alkyl chain is important in

governing the larvicidal activity of such phenylpropenes. *Della Greca et al.* [17], in their SAR studies of phenylpropanoids, have also observed that the presence of a MeO group at the aromatic ring enhanced the effectiveness of phenylpropanoids.

Scheme. *Comparative Benefits of Dihydrosafrole* (**16**) *over an Isomeric Mixture of Safrole*



In earlier studies, it was observed that OCH_2O substitution greatly enhanced the pesticidal activity of phenylpropenes [10][15]. This OCH_2O moiety is found in many natural compounds, *e.g.*, piperine alkaloids, from which the commercial synergist piperonyl butoxide (PBO) is derived, which interacts with the cytochrome P450 and inhibits the activity of the polysubstrate mono oxygenases (PSMOs) responsible for the metabolism of toxins in insects. In the present study, substitution with an OCH_2O group also enhanced the activity of phenylpropenes against the tested larvae. Interestingly, both isomeric forms, isosafrole (**6**) and safrole (**12**), demonstrated good activity (*Table 3*). In this case, the β-isomer **6** was 12.6 times more active than the γ-isomer **12** after 48 h of exposure, which is in accordance with various reports on higher activity of β-isomers [15][18][19]. The inconclusive observation regarding the trend of activity of phenylpropenes with respect to β- or γ-isoforms prompted us to reduce the C=C bond present in the alkyl side chains of phenylpropenes and to compare the activity of the resulting hydrogenated compounds, dihydroanethole (**15**), dihydrosafrole (**16**), dihydroeugenol (**17**), and dihydroasarone (**18**) with either of their isomeric counterparts. Among the tested hydrogenated compounds, only compound **16** showed promising activity with an LC_{50} value of 2.7 μg/leaf cm^2 after 48 h of exposure (*Tables 2* and *3*). It is pertinent to mention that phenylpropenes are vulnerable to oxidation at the C=C bond of the alkyl side chain when kept for a prolonged period of time [20]. However, the hydrogenated products **15**–**18**, devoid of oxidation susceptibility, with substantial retention of activity, have the advantage of an extended shelf life and storage potential (*Scheme*) and may be considered for their use in formulation with other promising phytochemicals.

Phenylpropenes can act in different ways against insects [18]. Although the mechanisms of toxicity of phenylpropenes against the lepidopteran larvae are not well known, several observations point to some physiological effects at the endocrine, enzymatic, and digestive level [18][21]. Some of the compounds under study, *i.e.*, β-

asarone (**8**), methyleugenol (**11**), and eugenol (**13**), showed delayed mortality, which could be attributed to several factors acting separately or together, *viz*., the uptake of the active moiety of the compound could be time dependent, leading to a progressive increase in the concentration of the compounds tested and their effect on the larval body, or the active moiety of the compound could be converted into more toxic metabolites in the larval integument and alimentary canal, resulting in time-dependent effects [22].

Several phenylpropenes, such as eugenol (**13**) [4][13][23], isoeugenol (**7**) [13], β-asarone (**8**) [24], anethole (**4**) [11][25], have been reported active against insects/mites. SAR of plant-derived compounds against arthropod pests have been well studied [26]. However, only limited studies on SAR of phenylpropenes against insects/mites have been reported [15][18][24][27][28], and no systematic studies are available on various structures of phenylpropanoids, particularly phenylpropenes, against *S. litura*. It has to be mentioned that a quantitative comparison of the toxicity of related phenylpropanoids is difficult with the phenylpropenes and some of their dihydroderivatives tested in the present study, due to different test insects and bioassay methods.

Conclusions. – The larvicidal activity and SAR of natural phenylpropanoids and their semisynthetic derivatives against *S. litura* were investigated, which has not been reported earlier. It was shown that OCH_2O substituted phenylpropenes have a promising insecticidal potential against *S. litura*. Isosafrole showed the highest activity (LC_{50} value of 0.6 μg/leaf cm^2 after 48 h) while its isomer safrole showed to be 12.6 times less active (LC_{50} value of 7.6 μg/leaf cm^2 after 48 h).

The problems associated with the separation of isomeric mixtures of phenylpropenes and, furthermore, susceptiblitity of the C=C bond of, *e.g*., safrole/isosafrole, with time towards air oxidation, resulting in the reduction of larvicidal activities, may be solved by hydrogenation of the isomeric mixtures. In this context, the activity shown by dihydrosafrole (LC_{50} value of 2.7 μg/leaf cm^2 after 48 h) is of interest, in particular in combination of its better stability and resistance to oxidative degradation in comparison to isosafrole/safrole. This observation may prove a pivotal point for further studies, and work in this direction is in progress. The incorporation of dihydrosafrole within insecticidal formulations could increase the number of biochemical targets in the insects, limiting the prospects for the onset of resistance and offering the means of reducing pesticide dosage due to possible synergistic or additive action.

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Experimental Part

General. 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ) was purchased from *Merck* (India). Column chromatography (CC): silica gel (SiO_2; 60–120 mesh). ^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance-300* spectrometer at 300 and 75.4 MHz, resp., in $CDCl_3$; δ in ppm, *J* in Hz. *CEM Discover* focused microwave (2450 MHz, 300 W) was used wherever mentioned.

Test Compounds and Their Synthesis. Compounds **3**–**7** and **10**–**13** were procured from commercial sources (*Merck* and *Sigma Aldrich*) and were used without any further purification. Compounds **1**, **2**, **9**,

14, and **15**–**18** were synthesized by the reported methods [29][30]. Compound **8** was isolated and purified from the essential oil of the rhizome of *Acorus calamus* by hydrodistillation using the method reported by *Sinha et al.* [31].

Methyl 4-Methoxycinnamate (**1**). A mixture of 4-methoxycinnamaldehyde (**2**; 18.75 mmol), DDQ (28.15 mmol), *Amberlyst-15* (0.1–0.2 g), MeOH (5 ml), and toluene (15 ml) in a round-bottom flask was irradiated in a focused monomode microwave system (100 W, 110°), fitted with a condenser and a *Dean–Stark* apparatus, for 40 min. The precipitated $DDQH_2$ was filtered, and the filtrate was passed over a bed of neutral alumina column and eluted with a 2–5% mixture of MeOH in toluene. The obtained org. layer, after evaporation under vacuum, provided **1** (90 % yield). White solid. M.p. 95°. ^{1}H-NMR: 7.67 (*d*, *J* = 16.15, 1 H); 7.40 (*d*, *J* = 8.48, 2 H); 6.83 (*d*, *J* = 8.48, 2 H); 6.26 (*d*, *J* = 16.15, 1 H); 3.75 (*s*, 3 H); 3.71 (*s*, 3 H). ^{13}C-NMR: 167.7; 161.4; 144.5; 129.7; 127.1; 115.2; 114.3; 55.3; 51.5. The spectral data matched well with the reported values [30].

4-Methoxycinnamaldehyde (**2**). A mixture of **15** (8.5 mmol), dry dioxane (50 ml), AcOH (2–4 drops), and DDQ (26.5 mmol) in a beaker was sonicated for 2 h or till disappearance of the starting material on the TLC plate. After completion of the reaction, the precipitated, solid $DDQH_2$ was removed by filtration, and the filtrate was evaporated. The residue was taken in AcOEt (50 ml) and washed with H_2O (2 × 10 ml), 2% $NaHCO_3$ (2 × 5 ml), and brine (2 × 10 ml), dried (Na_2SO_4), and filtered. The filtrate was evaporated to afford a crude yellow liquid, which was chromatographed on neutral alumina using hexane/AcOEt mixtures with increasing proportions of AcOEt up to 40% to provide **2** (70% yield). White solid. M.p. 58–59°. ^{1}H-NMR: 9.68 (*d*, *J* = 7.8, 1 H); 7.58 (*d*, *J* = 15.8, 1 H); 7.19–7.17 (*m*, 2 H); 6.72–6.70 (*m*, 2 H); 6.63 (*dd*, *J* = 15.8, 7.8, 1 H); 3.73 (*s*, 3 H). ^{13}C-NMR: 190.0; 161.2; 150.3; 129.6; 127.6; 127.2; 114.0; 56.0. The spectral data matched well with the reported values [29].

2,4,5-Trimethoxyphenylpropene (**9**). A mixture of **18** (4.8 mmol), DDQ (6.0 mmol), and SiO_2 (0.5–0.6 g) in anh. dioxane (50 ml) was stirred at r.t. for 14 h under N_2 till completion of the reaction. The precipitated $DDQH_2$ was filtered and the filtrate was evaporated and subsequently chromatographed on SiO_2 (hexane/AcOEt 7:3) to provide **9** (72% yield). White solid. M.p. 44–45°. ^{1}H-NMR: 6.91 (*s*, 1 H); 6.64 (*dd*, *J* = 1.5, 16.0, 1 H); 6.51 (*s*, 1 H); 6.02 (*dq*, *J* = 6.2, 16.0, 1 H); 3.84, 3.81, 3.77 (3*s*, 3 H each); 1.87 (*dd*, *J* = 6.2, 1.5, 3 H). ^{13}C-NMR: 151.0; 149.0; 142.6; 124.4; 123.4; 118.3; 109.2; 97.3; 56.1; 55.7; 55.1; 18.7. The spectral data matched well with the reported values [29].

Synthesis of **14**. A mixture of **13** (1.0 mmol), AcONa (4.0 mmol) and Ac_2O (4.0 mmol) was irradiated by microwave (100 W, 130°) for 40 s. After work up, **14** was obtained (98% yield). The spectral data matched well with the reported values [10].

Synthesis of **15**–**18**. Compounds **15**–**18** were prepared according to the method reported by *Joshi et al.* [29]. Compounds **4**, **6**, **7**, or **8**, resp., (15 mmol) were adsorbed on the powdered mixture of SiO_2 (4 g), palladium chloride (0.2 g), and ammonium formate (0.5 g), and 4 ml of formic acid (85%) and 5 ml of H_2O were added. The mixture was irradiated in a monomode microwave (100 W, 120°), fitted with a reflux condenser, for 8–10 min. Column purification of the crude mixture provided the corresponding hydrogenated derivatives (88–94% yield). The spectral data of the compounds matched well with reported values [29].

Test Insects. Insects, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae), were obtained from infested field crops, reared in the laboratory on *Ricinus communis* (Castor) leaves, and maintained at 23 ± 1°, 65 ± 5% rel. humidity, and a photoperiod of 16:8 (L:D). Second instar larvae, obtained from the established colony maintained for >20 generations in the laboratory, were used in the experiments.

Bioassays. Preliminary Screening. The larvicidal activity of **1**–**18** was evaluated by the leaf dip method [32] against 2nd instar larvae. Larvicidal activity was evaluated on castor leaves (34 cm^2) as a preferred host, since it shows strong attractiveness and palatability for *S. litura*. In addition, preference for castor was not overcome by exposure to novel deleterious chemicals [33]. It has also been shown that the effect of the host on the mid gut carboxyl esterase activity, compared to the one of *S. litura* fed on artificial diet, was not significant [34]. However, any possible effect of the induction of detoxification enzymes on the alteration of the toxicity of the test compounds were taken into account, as the data were corrected for the mortality of the untreated control insects using *Abbott*'s formula [35] whenever required.

A known amount of test compounds was dissolved in acetone and then diluted with H_2O to obtain the desired concentrations. Test compounds were suspended in distilled H_2O using *Triton X-100 LR* spreader (*S. D. Fine-Chem. Ltd*, India) at 0.1 ml/l. Preliminary screening of the test compounds was carried out at two high test dosages, *i.e.*, 10000 and 5000 ppm, corresponding to a concentration of *ca*. 30 and 15 μg/leaf cm^2, resp. Three leaf disks were separately dipped in each test soln. for 30 s and dried under the gentle stream of air under laboratory conditions. Second instar larvae (10 larvae in each replicate), starved for 3–4 h prior to the bioassay, were transferred individually on treated and control (disks treated with H_2O mixed with acetone/*Triton* only) leaf disks placed in *Petri* plates. All treated samples were maintained at $23 \pm 1°$, $65 \pm 5\%$ rel. humidity, and a photoperiod of 16:8 (L:D) in the laboratory. Mortality was determined 24 and 48 h after the larvae were placed on disks. Larvae not showing movements when probed with a camel hairbrush were considered dead.

Dose–Response Experiment. Based on the preliminary screening results, promising test compounds were selected and subjected to a dose-response bioassay. Test compound solns. (six concentrations each) were prepared as described above to provide dosage in the range of 0 to 30 μg/leaf cm^2. Commercial pesticides, a biological (*NeemAzal*, 0–6.0 μg /leaf cm^2) and a chemical (methyl parathion, 0–1.2 μg/leaf cm^2), commonly applied as *S. litura* controls, were used as positive controls.

Data Analysis. Mortality data were corrected, whenever required, for control mortality by using *Abbott*'s formula [35]. The concentration that killed 50% of the population relative to the control (LC_{50} value) was determined using SPSS 10.0 for Windows 2000 and Excel Microsoft program 3.

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