EVALUATION OF DIETARY TOXICITY OF SOME ESSENTIAL OIL ALLELOCHEMICALS FOR THE MANAGEMENT OF *CHILO PARTELLUS* (SWINHOE)

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Abstract: Eight essential oil compounds were used for study of larvicidal and growth inhibitory effects against the maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). It is the most prevalent key pests, and is one of the limiting factors in the successful cultivation of the maize crop in India. Two dietary bioassays; on-diet and in-diet, were used. The bioassays showed different tendencies and LC_{50} values were on the lower side in on-diet tests compared to in-diet assays. Thymol was the most active in both laboratory and greenhouse experiments. In greenhouse conditions, thymol was significantly active at a 10 x LC_{50} level of treatment. Overall mortality due to thymol was 41.66% in 4 days and larvae failed to bore into the plant and could not feed until 48 h post-treatment. After 72 h there was some degree of feeding and boring in the plants by insects, but still < 45% of that in the controls. The present study suggests that larvicidal and growth inhibitory activity by the essential oil compounds could be beneficial as a possible control of the maize borer. This is especially true about thymol which has potential in field situations. It is effective if a proper delivery system and the appropriate time of sprays is established. A 4 day-interval spray of thymol against *C. partellus* can control the pest significantly and can be used in any integrated pest management system. On the basis of compound structure, no specific trend was observed.

Key words: essential oil compounds, larvicides, growth inhibitors, maize borer, *Chilo partellus*

INTRODUCTION

The lepidopteran maize stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), is the most prevalent key pest and is one of the limiting factors in the successful cultivation of the maize crop, *Zea mays* (L.) (Poaceae). It is currently known to occur in India, Pakistan, Afghanistan, Nepal, Bangladesh, Sri Lanka, Thailand, Laos, Vietnam, Yeman and portions of Indonesia (CABI 2007). In Africa, it is found in most eastern and southern sub-Saharan countries, and there is one report in Australia (Ampofo and Saxena 1989). This insect is a leaf feeder. It causes tunneling within the stalk and disrupts the flow of nutrients to the ear. It causes subsequent development of deadhearts. Yield loss is attributed to the physiological effects on final ear size, lodging, or the complete loss of ears. Larval tunneling within the stalk may also predispose plants and ears to infection by fungal pathogens. Such infections further compromise the long-term storability, and quality of food products (Kfir *et al.* 2002). Yield loss estimates for maize stem borer vary greatly depending upon the country, season, maize variety and fertilization (De Groote 2002). Yield losses of 26.7 to 80.4% in different agroclimatic regions in India were recorded (Panwar 2005; Hari *et al*. 2008). Such an amount is a tremendous economic loss to farmers. In order to prevent such losses chemical insecticides were used as the main control agents from the early years of maize borer research (van den Berg 1992; Ahmad *et al*. 2007; Teli *et al*. 2007). However, the use of synthetic insecticides on corn fields led to potential adverse effects, including water and soil contamination, insect resistance and toxicity to non-target species. Integrated pest management (IPM) is now a well known practice. It is a practice receiving increased attention, which can help with management against corn borers (Ding *et al*. 1989; Lee *et al*. 1999). Plant derived compounds have made some impact in recent years as insecticides, antifeedants, and insect growth regulators (Dev and Koul 1997; Koul 2005; Koul and Walia 2009) and more specifically the use of compounds from essential oils (Koul *et al*. 2008).

Use of plant essential oils in pest management has gained momentum during the past decade. This is due to their eco-friendly properties and activity against a variety of insect pests of agricultural, public health and veterinary importance (Koul *et al*. 2008). Essential oils are abundantly found in plant families like: Apiaceae, Pinaceae, Lamiaceae, Myrtaceae, Rutaceae, Umbelliferae, Asteraceae, Annonaceae, Lamiaceae, Zingiberaceae and Lauraceae (Bekele *et al*. 1995; Mordue *et al*. 1998; Bekele

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and Hassanali 2001; Isman and Machial 2006; Akhtar *et al.* 2008; Regnault-Roger and Philogene 2008) and are the by-products of plant metabolism. Essential oils are commonly referred to as volatile plant secondary metabolites, which generate in response to stress conditions (Rice and Coats 1994). These allelochemicals interfere with metabolic, physiological and behavioral functions of insects. Some of these allelochemicals effect growth, development, reproduction or survival of insects and vectors (Brattsten 1983; Karr and Coats 1988; Wantanabe *et al*. 1993; Paruch *et al*. 2000; Tripathi *et al*. 2000; Koshier and Sedy 2001; Hummelbrunner and Isman 2001; Dimetry *et al*. 2003; Tripathi *et al*. 2002, 2003; Govindaraddi 2005; Koul 2005; Batish *et al*. 2008; Singh *et al*. 2008). Some plant essential oils also act as semiochemical disruptants (Youssef *et al*. 2009). The essential oil compounds display characterisitic neurotoxic symptoms including agitation, hyperactivity, paralysis and knockdown (Koul *et al*. 2008). The mode-of-action of these compounds, however, is not fully characterized. Many compounds are also widely used as flavoring agents in foods and beverages, food supplements, perfumes, decongestants and antiseptics (Templeton 1969). They are even exempt from pesticide registration. This special regulatory status combined with the wide availability of essential oils from the flavor and fragrance industries, has made it possible to fast-track commercialization of essential oil-based pesticides.

There is an absence of strategy for the use of eco-friendly compounds against the maize stem borer, *C. partellus*. This lack of strategy extends mainly to the use of essential oil compounds from plant sources in an integrated pest management system. The objective of the present study was to evaluate the dietary toxicity of such compounds and determine the influence on the survival, growth and development, pupation rate, and adult emergence rate of the maize stem borer, *C. partellus*. We also observed the differences in results from two different application methods, i.e. diet incorporation or application onto the surface of the diet. We tried laboratory results in greenhouse experiments to determine if an IPM based strategy could be developed.

MATERIALS AND METHODS

Test compounds

We used eight essential oil compounds, i.e. thymol, 1,8-cineole, linalool, terpineol, *trans*-anethole, carvacrol, eugenol and methyl eugenol, in the present study. The compounds occur commonly in mint, myrtle, citrus, eucalyptus, rosemary etc. We used the pure compounds (97–99% purity) as received (purchased from Sigma/Aldrich Chemie, Gmbh, Germany and Acros Organics, NJ).

Insects

Bioassays were conducted using larvae of the maize borer, *C. partellus* obtained from an established laboratory colony. Larvae were maintained on an artificial diet prepared in the laboratory (Kanta and Sajjan 1992). The cultures were maintained at 27±1°C at L16 : D8 photoperiods. First and second instars were used in various experiments.

In-diet bioassay

Lethal concentrations (LC_{50}) which cause 50% mortality and effective concentrations (EC_{50}) that inhibited 50% growth were determined by oral administration methods. These were the concentrations required for 1st and 2nd instars of *C. partellus* larvae. Various compounds were dissolved in solvent (acetone) to prepare a stock solution. Required quantity from the stock solution was then picked and individually mixed with the standard artificial diet before the diet solidified. For example, a prepared concentration of the chemical solution was incorporated into the diet when the dietary preparations cooled down to ~40°C. This was done to prepare a series of concentrations in the range from 0.05 to 1.5 mg/ml for the 1st instars and 0.5 to 8 mg/ml for the 2nd instars. The mixtures were then thoroughly blended. The artificial diet was carried in a warm jar. Running hot water maintained the temperature at ~58°C to prevent hardening. Keeping the optimum temperature is very important. Higher temperatures can degrade some ingredients such as vitamins, and low temperatures can allow the medium to solidify too quickly. The control diet was treated with solvent only. Upon hatching, single 1st (48 h old) and 2nd (7–9 mg body weight) instars were placed individually on ~3 g diet poured into individual solo cups (29.57 ml) as larvae have a boring habit. Larvae were individually weighed prior to treatment. Treatment groups were then placed in a sealed plastic tray lined with moistened filter paper and held in a growth chamber (L16 : D8, 26°C and 75±2% RH). The tray containing *C. partellus* larvae was covered on the upper side by a black paper sheet because larvae have positive phototropism. The larval growth was assessed as a percentage of the controls after 7 days based on larval weight. A concentration inhibiting 50% growth relative to the controls, was determined by regression analysis. Total larval mortality was also recorded after 7 days for each treatment, by counting the number of dead larvae. LC_{50} required for 1st and 2nd instars was determined by probit analysis. Twenty four larvae were used for each concentration. Data on consumption of diet was also recorded for each treatment to assess percent reduction in diet consumed over the control.

On-diet bioassay

We designed this test to determine the differences between the two chemical application methods. In this bioassay a 7.5 g artificial diet was poured into each solo cup (29.57 ml), and the dietary preparations were allowed to solidify at room temperature (25±2°C). Serial dilutions of each compound ranging from 0.05 to 1.7 mg/ml were prepared by using certified acetone as described earlier. An appropriate amount (200 µl per cup) from each concentration was applied on the surface of the diet (~3 cm diameter) in an individual cup. The control diet was treated with acetone only. The cups were open for 1 h to allow the solvent to evaporate. One 1st instar larva (48 h old) was placed individually with a fine brush on the center of each diet treated with a compound at various concentrations. Treatment groups were then placed in a sealed plastic tray as described earlier. Total larval mortality was recorded after 7 days for each treatment, by count-

ing number of dead larvae. LC_{50} required for 1st instar *C. partellus* larvae was determined by using probit analysis. Twenty four larvae were used for each concentration.

Bioassay for developmental effects

Any developmental effects of essential oil compounds on larval period, percent pupation, pupal weight, pupal period, sex ratio (male : female) and adult emergence of *C. partellus* was determined at EC_{50} levels of concentration. For this, the required concentrations were prepared by using acetone as a solvent, and mixed with the diet as described earlier. One 2nd instar larva (7–9 mg body weight) was placed in an individual solo cup containing the treated diet. Treatment groups were then placed in a sealed plastic tray as described earlier. The diets of respective cups were changed every 7 days, and any mortality caused was recorded. Twenty eight larvae were used for each concentration. Various parameters were calculated as under:

1. Larval period: the larval period was taken as the interval (days) between the date of release of a 2nd instar larva and formation of its pupa.
2. Pupation: the pupation percent was determined as:

$$\text{Pupation}(\%) = \frac{\text{total number of pupa formed}}{\text{total number of larvae released}} \times 100$$

3. Pupal period: the pupal period was calculated as the interval between pupal formation and the emergence of the adult.
4. Pupal weight: the weight of each pupa (mg) was calculated.
5. Sex ratio: as the larvae reached the adult stage, they were examined for their sex and thus sex-ratio was determined.
6. Adult emergence:

$$\text{Adult emergence}(\%) = \frac{\text{total number of adults formed}}{\text{total number of pupae}} \times 100$$

Greenhouse evaluation

A greenhouse evaluation of the most active compounds found in the laboratory experiments was conducted. The evaluation was to determine the larval mortality percent, percentage of larvae that bored into the whorl of the plant, and total area (mm^2) consumed by the larva per plant.

The second instars were used in this experiment and the required concentrations used in the study were based on LC_{50} values determined in the laboratory experiments (Table 2). The treatments were given at LC_{50} level and the multiples of 5 and 10 times this value. The greenhouse studies were conducted on maize plants (Cultivar "Kesri", recommended by Punjab Agricultural University, Ludhiana) against *C. partellus*, larvae. The plants (1 plant per pot) were sown in pots of 15 x 10 cm size. All packages and practices for maize crop, recommended by Punjab Agricultural University, Ludhiana were followed.

Each desired concentration was prepared using acetone and water (1 : 15 ml). For spraying purposes, Triton X 100 @ 0.2% was added as an emulsifier. Each plant was sprayed with a hand sprayer (JSGW, Ambala, India) of 100 ml capacity to run off (12–15 ml solution). The experiments were conducted on 21-days-old plants. In experiments, each pot was covered with a nylon net, which was tied with a thread from the bottom. In each concentration there were 12 replicates. Data were taken at different time intervals i.e., 24, 48, 72, and 96 h after spraying and cumulated after 96 h. Spraying was done after releasing the larvae in the centre of a whorl. There were 12 larvae per plant. After treatment the pots were kept at an ambient temperature of 28±1°C, RH 80% and L16 : 8D photoperiod.

Data analysis

Statistical analysis was performed using Probit analysis to determine LC and EC values with their 95% confidence limits (Finney 1971). Developmental parameters were compared with ANOVA followed by Tukey's multiple range tests to determine differences between means of treatment (SAS 2002).

RESULTS

Larvicidal toxicity

The compounds evaluated for this study showed some degree of larvicidal activity when they were incorporated into the diet (in-diet test) or when applied on the solidified diet surface (on-diet test). Most larvicidal activity occurred between 4 and 6 days after treatments, therefore mortality data from daily observations were accumulated until the 7th day. Significant differences were recorded among various compounds according to LC_{50} and 95% CL.

In-diet assays were carried out for both the first and second instars. Larval mortality of the first instars was higher when applied in-diet (Table 1). Within the group of compounds tested, thymol was most active (LC_{50} = 0.11 mg/ml), followed by 1,8-cineole (LC_{50} = 0.13 mg/ml) and linalool (LC_{50} = 0.16 mg/ml). The other compounds (terpineol, carvacrol, *trans*-anethole, eugenol and methyl eugenol) were less toxic (LC_{50} = 0.38–0.78 mg/ml) than thymol and 1,8-cineole.

All the essential oil compounds selected for the study showed some degree of larvicidal activity when compounds were applied on the solidified diet surface (on-diet assay). Significant differences occurred (after 7 days) among essential oil compounds according to LC_{50} values. Larval mortality of 1st instars, due to thymol in this experiment, was 0.19 mg/cup (LC_{50}) after 7 days followed by 1,8-cineole and linalool with LC_{50} values of 0.22 and 0.26 mg/cup, respectively. Other compounds terpineol, *trans*-anethole, carvacrol, eugenol and methyl eugenol were 3 to 4-fold less toxic with LC_{50} values in the range of 0.60 to 0.96 mg/cup with methyl eugenol being the least toxic compound (Table 1).

In the case of 2nd instars, where an in-diet assay was conducted, thymol was again the most toxic compound with LC_{50} = 2.08 mg/ml. Terpineol and 1,8-cineole were significantly similar which was obvious by the overlap of confidence intervals (Table 2). Linalool (LC_{50} = 2.68 mg/ml) was moderate in comparison to thymol and terpineol.

Table 1. Larvicidal toxicity (after 7-days) of essential oil compounds incorporated into and on the surface of artificial diet against 1st instar *C. partellus* larvae

Compounds	In-diet bioassay			On-diet bioassay		
	LC_{50} [mg/ml]	95% CI	slope	LC_{50} [mg/cup]	95% CI	slope
Thymol	0.11	0.09–0.13	2.18±0.26	0.19	0.16–0.22	2.06±0.21
1,8-cineole	0.13	0.09–0.20	2.08±0.47	0.22	0.16–0.30	1.97±0.35
Linalool	0.16	0.10–0.25	2.04±0.42	0.26	0.20–0.35	1.90±0.28
Terpineol	0.38	0.27–0.55	2.40±0.61	0.60	0.50–0.72	3.04±0.49
Trans-anethole	0.49	0.30–0.80	1.96±0.6	0.72	0.57–0.91	2.43±0.47
Carvacrol	0.54	0.42–0.70	2.60±0.57	0.70	0.55–0.89	3.16±0.79
Eugenol	0.63	0.53–0.76	2.97±0.50	0.85	0.79–0.90	4.33±0.33
Methyl-eugenol	0.78	0.66–0.93	4.70±1.05	0.96	0.82–1.11	4.42±0.87

Table 2. Larvicidal toxicity (after 7-days) of essential oil compounds incorporated into the artificial diet against 2nd instars of *C. partellus* larvae

Compound	LC_{50} [mg/ml]	95% CI	Slope
Thymol	2.08	1.77–2.44	4.02±0.74
1,8-cineole	2.54	2.19–2.94	3.42±0.57
Linalool	2.68	2.37–3.02	4.39±0.67
Terpineol	2.55	2.24–2.90	4.28±0.75
Trans-anethole	3.22	3.00–3.45	5.38±0.55
Carvacrol	3.66	3.24–4.14	4.06±0.56
Eugenol	3.87	3.43–4.36	4.97±0.80
Methyl-eugenol	4.34	3.70–5.10	3.85±0.75

Table 3. Effective concentrations of essential oil compounds that inhibited growth (% of controls) of *C. partellus* larvae, when incorporated into the artificial diet

Compounds	1st instars			2nd instars		
	EC_{50} [mg/ml]	95% CI	slope	EC_{50} [mg/ml]	95% CI	slope
Thymol	0.07	0.06–0.08	2.17±0.19	1.41	1.17–1.69	2.71±0.49
1,8-cineole	0.08	0.07–0.10	1.45±0.19	1.70	1.49–1.94	3.03±0.41
Linalool	0.13	0.10–0.15	2.50±0.46	1.95	1.79–2.12	3.14±0.31
Terpineol	0.25	0.23–0.27	3.34±0.39	2.23	2.09–2.38	4.45±0.52
Trans-anethole	0.27	0.23–0.34	3.72±0.62	2.38	2.13–2.67	2.43±0.22
Carvacrol	0.27	0.26–0.29	4.30±0.40	2.55	2.42–2.69	4.52±0.24
Eugenol	0.34	0.03–0.37	2.52±0.34	2.76	2.50–3.04	2.83±0.43
Methyl-eugenol	0.39	0.36–0.43	3.06±0.25	3.35	2.80–3.99	3.09±0.58

The other compounds were toxic in the range of 3.22–4.34 mg/ml. Methyl eugenol, however, was the least active compound (Table 2).

Determination of growth inhibition

The initial diet bioassay against 1st instars (2-day-old) using various essential oil compounds at different concentrations was to assess growth inhibition after 7 days post-treatment. There was significant growth inhibition that rose with an increase in the concentration of the compound. The effective concentration of essential oil compounds to inhibit 50% growth (EC_{50}) against *C. partellus* was significantly high with thymol (0.07 mg/ml) followed by 1,8 cineole (0.08 mg/ml) and linalool (0.13 mg/ml). Terpineol, *trans*-anethole and carvacrol were active between a 0.25 and 0.39 mg/ml (EC_{50}) level of treatment. Eugenol (EC_{50} = 0.34 mg/ml) and methyl eugenol (EC_{50} = 0.39 mg/ml) were least active in inducing growth inhibition among 1st instar *C. partellus* larvae.

Treatment to 2nd instars showed that 10 to 20 times more compound was required based on the EC_{50} values obtained against 1st instars (Table 3) to achieve a similar level of growth inhibition. Thymol was significantly the most active compound with an EC_{50} value of 1.41 mg/ml followed by 1,8-cineole and linalool with EC_{50} values of 1.70 and 1.95 mg/ml, respectively. Terpineol, *trans*-anethole, carvacrol and eugenol were moderately active with an EC_{50} values in the range of 2.23–3.35 mg/ml concentrations (Table 3). Methyl eugenol was the least active compound to inhibit growth of larvae.

Table 4. Effect of various compounds on the development of *C. partellus* in laboratory experiments at EC_{50} levels of treatment

Compound	Developmental parameters	Treatment	Control
Thymol	larval period [days]	24.4 b	18.2 a
	pupation [%]	64.28 b	100 a
	pupal period [days]	6.1 b	3.8 a
	pupal weight [mg]	male female 14.2 c 25.7 d	male female 21.3 a 36.4 b
	sex-ratio	1 : 0.63	1 : 1.54
1,8-cineole	larval period [days]	23.9 b	18.2 a
	pupation [%]	71.42 b	100 a
	pupal period [days]	5.8 b	3.8 a
	pupal weight [mg]	male female 14.8 c 27.3 d	male female 21.3 a 36.4 b
	sex-ratio	1 : 0.66	1 : 1.54
Linalool	larval period [days]	23.4 b	18.2 a
	pupation [%]	75.0 b	100 a
	pupal period [days]	5.3 b	3.8 a
	pupal weight [mg]	male female 15.6 c 27.9 b	male female 21.3 a 36.4 b
	sex-ratio	1 : 0.61	1 : 1.54
Terpineol	larval period [days]	22.4 b	18.2 a
	pupation [%]	78.57 b	100 a
	pupal period [days]	5.1 b	3.8 a
	pupal weight [mg]	male female 16.6 c 28.6 d	male female 21.3 a 36.4 b
	sex-ratio	1 : 0.83	1 : 1.54
Anethole	larval period [days]	22.1 b	18.2 a
	pupation [%]	85.71 b	100 a
	pupal period [days]	4.5 b	3.8 a
	pupal weight [mg]	male female 16.9 c 29.8 d	male female 21.3 a 36.4 b
	sex-ratio	1 : 1	1 : 1.54
Carvacrol	larval period [days]	21.4 b	18.2 a
	pupation [%]	85.71 b	100 a
	pupal period [days]	4.2 b	3.8 a
	pupal weight [mg]	male female 17.2 c 30.1 bc	male female 21.3 a 36.4 b
	sex-ratio	1 : 1.18	1 : 1.54
Eugenol	larval period [days]	21.1 b	18.2 a
	pupation [%]	89.28 b	100 a
	pupal period [days]	4.3 b	3.8 a
	pupal weight [mg]	male female 18.9 c 30.9 bc	male female 21.3 a 36.4 b
	sex-ratio	1 : 1.08	1 : 1.54
Methyl-eugenol	larval period [days]	20.4 b	18.2 a
	pupation [%]	89.28 b	100 a
	pupal period [days]	4.1 b	3.8 a
	pupal weight [mg]	male female 19.1 c 31.6 b	male female 21.3 a 36.4 b
	sex-ratio	1 : 1.27	1 : 1.54

All values are means of replicates. Means within a row followed by the same letter are not significantly different (ANOVA followed by Tukey's test; p = 0.05)

Developmental effects

The time taken by the 2nd instar larvae of *C. partellus* to reach the pupal stage, is presented in table 4. Significant results were recorded on the prolongation of larval period, when reared from the 2nd instar stage till pupation on diets treated at EC_{50} levels of efficacy of various compounds used in the present study. There was a very moderate effect on the consumption of diet by larvae, which was not significantly different ($p > 0.05$, ANOVA). Development was completed faster on an untreated diet as compared to treated diets. While on control diets it took about 18.2 days to complete the larval period. Under treated conditions it took 24.4 days depending upon the compounds used in the treatment (Table 4). There was prolongation of the pupal period as well as reduction in pupal weight. However, methyl eugenol again, was the least effective compound with minimal effect on various developmental parameters. The data revealed that a significantly more number of male adults were produced than females from *C. partellus* larvae, when reared on treated diets at EC_{50} concentrations of thymol, 1,8-cineole, linalool and terpineol. When larvae were reared on control diets, the male : female ratio of *C. partellus* was 1 : 1.54 (i.e. the number of female adults were more) (Table 4).

Results showed that ~90% adults of *C. partellus* emerged from pupae reared on diets treated with thymol, linalool and 1,8-cineole at EC_{50} level of treatment. The emergence of adults in the case of terpineol, carvacrol, *trans*-anethole, eugenol and methyl-eugenol were not significantly different from the controls.

Greenhouse evaluation

When thymol treatments were sprayed on the plants under greenhouse conditions there were significant results in percent larval mortality, amount of leaf area consumed (mm^2) and number of larvae that bored into the plant. In control plants, where only solvent mixture was sprayed, no mortality of larvae was observed after four days of spray. The number of larvae that bored into the plant was 47.22% and total feeding area of leaves per plant recorded was 48.06 mm^2 during 4 days of observations. Thymol was again the most active compound in the highest treatment (10 x LC_{50}) used that caused 41.7% mortality in up to 96 h (Fig. 1). No larvae could bore into the plant before 72 h. There was some feeding and boring observed after 72 h, which was still less than 45% of controls. At lower concentrations of thymol (5 x LC_{50}), a 25.2% mortality was recorded and no larva was seen boring into the plant for the same duration. These values remained almost constant for 96 h of treatment (Fig. 1).

The mortality due to 1,8-cineole and linalool in a similar type application at their highest concentration, was around 20%, and 38.8% larvae bored into the plants in 96 h. Feeding was also significantly low as compared to the controls (Fig. 1) but more than observed in thymol treatments.

A ten time LC_{50} concentration spray was applied on plants using terpineol. Larval mortality, larvae that bored into the plant and total feeding area consumed per plant were 19.44%, 44.44% and 26.89 mm^2, respectively, 96 h after the sprays were applied.

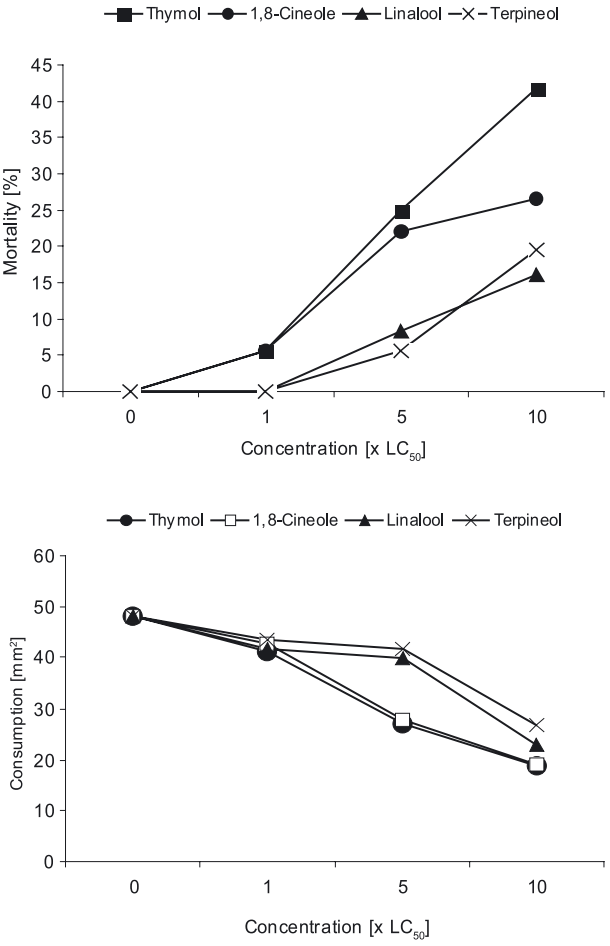

Fig. 1. Mortality of *C. partellus* larvae and effect on the consumption after a 96 h exposure to corn plants treated with essential oil compounds under greenhouse conditions

DISCUSSION

Many essential oil compounds were studied and cause a variety of effects in insects (Koul 2008). But their spectra of activity and modes of action as regulators of growth and development are poorly understood. The present work focuses on the potential of such compounds acting via ingestion. Laboratory dietary bioassays conducted, showed that selected compounds had various biological effects against the maize borer, *C. partellus*. The compounds used, showed larvicidal activities against the maize borer from a moderate to a high degree. Among the test compounds, thymol was most active, followed by 1,8-cineole and linalool. Eugenol and methyl eugenol were least active as compared to other compounds. Thymol is apparently a very potent larvicide as it is toxic to other lepidopterans like *Spodoptera litura* (Hummelbrunner and Isman 2001) and *Thaumetopoea wilkinsoni* (Cetin *et al*. 2007). Currently, five end-use pesticide products containing the active ingredient thymol are registerd. Thymol, thyme essential oil and thyme (spice) are listed by the Food and Drug Administration (FDA) as foods for human consumption and food additives.

On-diet and in-diet assays showed slightly different tendencies as the compounds were administered in two different ways. LC_{50} values were on the lower side in on-

diet tests compared to in-diet tests against first instars (Table 1), suggesting that efficacy in on-diet experiments was lower. The obvious reason for this could be that *C. partellus* larvae did not feed on the same amount of the compound at the surface of the diet due to their boring into the diet where compounds were not available, as would be in the in-diet experiment. In, the in-diet experiment insects have to feed continuously on the treated diet. This has an implication in the field situation in the sense that sprays on the plants would be required before the larvae reach to the boring stage of development. This means that treatments should be given before late second instars, which have tendency to bore into 20–30 day old plants. The comparison of 1st and 2nd instars for the in-diet experiments revealed that 1st instars were more susceptible to all the compounds than the second instars. Nearly a 5 to 18-fold increase in concentration level was required to achieve similar toxicity in 2nd instars.

Thymol is definitely the most active. The other monocyclic compound α-terpineol has almost half of the activity of linalool (acyclic) against *C. partellus* larvae. On the basis of the structure of the compounds used in the present study, there does not seem to be any specific trend. This is despite the fact that earlier reports suggest that monocyclic monoterpenoids were generally more effective than acyclic and bicyclic compounds against *Ostrinia nubilalis* in dietary assays (Lee *et al*. 1999) and *Diabrotica undecimpunctata* in a soil assay (Rice and Coats 1994).

1,8-cineole was the second best effective compound against *C. partellus* and this compound is shown to have potential against other lepidopterans like *S. litura, Psedaletia unipuncta* and *Trichoplusia ni* (Hummelbrunner and Isman 2001; Isman *et al*. 2008). Linalool was significantly similar to thymol and 1,8-cineole in its activity against *C. partellus* and is known as a potent, efficacious compound against mosquitoes (Traboulsi *et al*. 2002, 2005; Cheng *et al*. 2009) and fruit flies (Chang et al. 2009), however, moderate activity has been reported against red bud borer, *Resseliella oculiperda* (van Tol *et al*. 2007).

Investigation of growth inhibitory effects showed that all the compounds did inhibit the growth at variable concentrations of the compounds used. Thymol was again very effective in inhibiting the growth of both 1st and 2nd instars (EC_{50} = 0.07 and 1.41 mg/ml, respectively). Inhibition of larval growth due to thymol has been reported for *S. litura* earlier after topical application (Hummelbrunner and Isman 2001). The maximum pupation rate was affected by thymol (35%), where as other compounds affected pupation between 10 to 15%. Pupal period, however, did increase in all cases in comparison to the controls. This suggests that these pests would complete fewer generations, multiply less quickly and would remain exposed to natural enemies for a longer period. This would supply natural control. These factors will cause less damage and will help increase crop yield.

While determining the sex ratio under both treated and untreated conditions, it was observed that normally more females were produced than males in controls (sex ratio: 1 male : 1.54 females). In the case of thymol, 1,8-cineole and linalool treatments the sex ratio was the reverse of the sex ratio in the controls, i.e. high proportion of males were produced. This is interesting and should favour the decrease in population build up. The reduction in number of females has great significance in the field, for depletion of pest populations.

Studies on carvacrol efficacy against lepidopterans are very scanty. Larvicidal activity, though, was determined against dipterans (Lee *et al*. 1997; Park *et al*. 2008) and stored grain pests (Ahn *et al*. 1998), and antifeedant activity against thrips was reported (Sedy and Koschier 2003). Carvacrol is larvicidal to *C. partellus* but to get a 50% kill of the population, up to a 5 times stronger concentration than that of thymol was required. The only other reports to show chronic toxicity of carvacrol against lepidopterans is against *S. litura* when applied topically (42.7 μg/larva). This form of application is approximately 1.7 fold less active than thymol reported against the same species (25.4 μg/larva) (Hummelbrunner and Isman 2001) and *Plutella xylostella* when applied via filter paper diffusion method (Ahn *et al*. 1998). Eugenol and methyl eugenol toxicity was comparatively moderate against *C. partellus* when compared to other compounds. Both these compounds are known to be attractants to fruit flies, particularly methyl eugenol used in cuelure traps (Vargas *et al*. 2005).

The four most effective compounds, thymol, 1,8-cineole, linalool and α-terpineol were evaluated in greenhouse conditions against *C. partellus* larvae at 5 and 10 times the LC_{50} concentrations used in the laboratory. Thymol was significantly active at the highest concentration used. At this concentration, overall mortality was 41.66% and larvae failed to bore into the plant and could not feed until 72 h post-treatment. After 72 h there was some degree of feeding and boring in the plants by insects, but still < 45% of that of the controls. This implies that these treatments could protect the maize crop successfully from attack of *C. partellus* 2nd instars for approximately 4 days and subsequent sprays will be required after this period to keep the plants completely from insect attack. 1,8-cineole was also active in a field situation but less than thymol, however, linalool and terpineol were very moderate in activity when compared to the controls in greenhouse experiments.

It can be concluded from the present study that larvicidal and growth inhibitory activity by the essential oil compounds could be beneficial as possible control of the maize borer. This is true specifically of thymol which has shown potential in both laboratory and greenhouse experiments in the present study. If a proper delivery system and the appropriate time of sprays are established, the pest can be controlled significantly. For example, a 4 day-interval thymol spray against *C. partellus* can control the pest significantly and can be used in any integrated pest management system. More studies are required at the field level to establish their efficacy on a larger scale.

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POLISH SUMMARY

OCENA TOKSYCZNOŚCI POKARMOWEJ WYBRANYCH ALLELOZWIĄZKÓW OLEJKÓW ETERYCZNYCH DLA *CHILO PARTELLUS* (SWINHOE)

Osiem olejków eterycznych badano pod względem efektywności larwobójczej i inhibującej przeciwko szkodnikowi *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae), powodującemu znaczne straty w uprawach kukurydzy w Indiach. Zastosowano dwa biotesty pokarmowe: „on--diet" oraz „in-diet". Wyniki przeprowadzonych testów wykazały zróżnicowanie, a wartości LC_{50} były niższe dla „on-diet" testów w porównaniu do ocen w „in-diet" testach. Olejek tymolowy okazał się najbardziej skuteczny zarówno badaniach laboratoryjnych jak i w doświadczeniach szklarniowych. W warunkach szklarniowych składnik aktywny tymol wykazywał wysoką skuteczność na poziomie traktowania 10 x LC_{50}. W wyniku zastosowania tymolu całkowita śmiertelność szkodnika wynosiła 41,66% po 4 dniach, a larwy utraciły zdolność do penetracji łodyg kukurydzy i żerowania aż do 48 godzin po zabiegu. Po upływie 72 godzin obserwowano pewien stopień żerowania i penetracji, jednak poniżej 45% w porównaniu do kontroli. Wyniki przeprowadzonych badań wykazały larwobójcze i inhibujące działanie olejków eterycznych oraz ich przydatność do zwalczania szkodnika kukurydzy *Chilo partellus*, a zwłaszcza w odniesieniu do składnika aktywnego – tymolu i jego potencjalnych możliwości wykorzystania w warunkach polowych. Olejek tymolowy wykazuje skuteczność przeciwko *Chilo partellus* pod warunkiem, że zabiegi są przeprowadzane prawidłowo w odpowiednim terminie, dlatego też może być włączony do programu integrowanej ochrony przed szkodnikami.