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Phytochemical analysis and biological activities of solvent extracts of *Ocimum basilicum* leaf and *Curcuma longa* rhizome and its effect on *Disonycha xanthomelas*

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Abstract
The study was conducted in Coimbatore, Tamil Nadu. In this study an attempt was made to control *Disonycha xanthomelas using Ocimum basilicum* (leaf) and *Curcuma longa* (rhizome) solvent extracts. From the phytochemical analysis of methanol extracts of *Ocimum basilicum* leaf and *Curcuma longa* rhizome determined the presence of Alkaloids, Flavonoids, Tannins, Saponins, Glycosides, Steroids, Terpenoids, Phenols, Quinone, and Phlobotannins. In antifeedant analysis the mortality rate of *Disonycha xanthomelas* is increased on the basis of Petroleum ether and methanol extract concentration. After 24 to 72 hours treatment the mortality rate percentage of *Disonycha xanthomelas* were 3.00% to 6.66% in petroleum ether extract of *Ocimum basilicum* leaf and 3.60% to 6.00% were observed in *Curcuma longa* rhizome extract. In methanol extracts of *Ocimum basilicum* leaf and *Curcuma longa* rhizome showed 2.33% to 6.00% and 2.30% to 6.00%. *Ocimum basilicum* leaf extracts in both solvents are slightly more effective than petroleum ether and methanol extracts of *Curcuma longa* against *Disonycha xanthomelas*.

Keywords: *Disonycha xanthomelas*, *Ocimum basilicum*, *Curcuma longa*

Introduction
The insecticidal properties of a number of plants have been discovered long ago. Botanical plant extracts are environmentally less harmful than synthetic pesticides to control pests. They possess one or more useful properties such as biodegradability, broad spectrum of activity and ability to reduce insect resistance. In present project work, plant extracts such as *Ocimum basilicum* and *Curcuma longa* have been tested for their phytochemical antifeedant, antimicrobial and GC-MS analysis against *Disonycha xanthomelas*.

Materials and Methods
The leaves of *Ocimum basilicum* and *Curcuma longa* rhizome were collected from, in and around Kerala. Collection was done from May to August. The collected leaves and rhizome were washed under running water and remove dirt materials. Soft stem was separated from the leaves manually during nibbing. Care was taken to avoid bruised and discoloured leaves. After the same procedure the rhizome of *Curcuma longa* rhizome were boiled for 45 to 60 minutes and were sun dried for 10 – 15 days. Leaves were shade dried and powdered using a pulveriser. The powder samples were stored in an air tight container for further study.

Preparation of extracts and fractionation
Extracts of different samples were prepared according to methods developed by Wagner H *et al*., Khandelwal KR. and Mukherjee PK.

Extraction using Soxhlet apparatus
The solvents used for the extraction procedure were Acetone, chloroform, ethanol, hexane, methanol, petroleum ether. About 50gm of dried plant powder of each sample was extracted with 250 ml of the extraction solvent using Soxhlet apparatus. The extracts were concentrated to dryness to yield crude residue. The extracts were autoclaved, labelled and stored at 4°c in air tight bottles. These residues were used for Phytochemical, anti-microbial activity against tested organisms, anti-oxidant activity and for other tests. Best yield of residue 25 was obtained in methanol hence for fractionation methanolic extracts was used.

For anti-oxidant activity the shade dried coarse powder of the basil leaf and rhizome of Turmeric was extracted using methanol and petroleum ether.

Phytochemical analysis
Preliminary Qualitative phytochemical Analysis was carried out to identify the secondary metabolites (Alkaloids, flavonoids, tannins, saponins, glycosides, steroids, terpenoids, phenols, quinone, and Phlobotannins) present in the various methanolic and petroleum ether extracts.

Detection of Alkaloids
The extract was dissolved in 1 ml dilute sulphuric acid and filtered using Whatman No.1 filter paper and the filtrate was treated with Mayer's reagent. The appearance of cream precipitates in response to the above reagents indicates the presence of alkaloids.

Detection of Flavonoids
Extract mixed with 1-2 ml of ammonia solution and then add 1 ml concentrated sulphuric acid. Appearance of yellow colour indicates the presence of flavonoids.

Detection of Tannins
Extract dissolved in 10 ml of distilled water and allow to settle and filtered. To the filtrate 1- 2 ml of 5% ferric chloride was added. Appearance of deep green colour indicates the presence of tannin. Another portion of filtrate was treated with 1-2 ml of iodine solution. Getting a faint bluish colour confirmed the presence of tannin.

Detection of Saponins
1 ml of the test extract dissolved in 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

Detection of Glycosides
Extracts were dissolved in 10 ml of distilled water under boiling conditions. This filtered and 2 ml of the filtrate was hydrolysed with a few drops of concentrated HCl and the solution was rendered alkaline with 1-2 drops of ammonia solution. Five drops of this solution were added to 2 ml of Benedict's qualitative reagent and boiled. A reddish-brown precipitate indicates the presence of glycosides.

Detection of steroids
Extracts were dissolved in 2 ml of chloroform and to this 2 ml of concentrated sulphuric acid add carefully added to form a lower layer. A reddish-brown colour at the interface indicates the presence of steroids.

Detection of Terpenoids
Extract dissolved in 1 ml of chloroform and 1 ml of acetic anhydrase. To this solution 2 drops of concentrated sulphuric acid were added. A pink colour which changes to bluish green on standing indicates the presence of terpenoids.

Detection of Phenols
Extracts were dissolved in 10 ml of water and ferric chloride solution (5%), or gelatin solution (1%) or lead acetate solution (10%). Appearance of deep blue colour with ferric chloride or precipitation with other reagents indicates the presence of total phenol.

Detection of Quinone
2 ml of test extract added to 3 ml of HCl. The appearance of a yellow precipitate indicates the presence of quinone.

Detection of Phlobotanin
2 ml of extract was added to 2ml 1% HCl. Formation of red precipitate indicates the presence of phlobotanin.

Antifeedant Test
The adult insects were chilled for a period of 12 minutes. The immobilized insects were picked up individually by using a small forceps. Solutions of different amounts of extractions (5.0, 10.0, 15.0, 20, 25.) were taken in a petri dish using pipette. Then transferred 15 test insects into the petri dish. Test insects were examined daily and those that did not move or respond to gentle touch were considered as dead. Insect mortalities were recorded at 24, 48, and 72 hours after treatment (HAT). Observed mortalities of the insects were corrected by One- way ANOVA. Forty-five insects, in three replicates of 15 insects each, were treated at each amount for the control test.

$$\text{Percentage of Antifeedant index} = \frac{C - T}{C + T}$$

Result and Discussion
Phytochemical analysis
The results of phytochemical analysis of two solvent extracts of *Ocimum basilicum* (*O. basilicum*) leaf and *Curcuma longa* (*C. longa*) rhizome were summarized in Table 1. Various bioactive molecules were found in *O. basilicum* leaf extract and *C. longa* rhizome extracts.
Petroleum ether extracts of *O. basilicum* showed the presence of Glycosides, Flavonoids, Phenols, Saponins, Terpenoids, Steroids and Tannins. The rest were not present. Whereas, Alkaloids, Flavonoids, Tannins, Saponins, Glycosides, Steroids Terpenoids, Phenols, Quinone, Phlobotannin were present in methanolic extracts of *O. basilicum* leaf.
In Petroleum Ether extract of C. Longa Quinone seems to be absent except Glycosides and Quinone all other secondary metabolites were present in Methanolic extracts of *C. longa* rhizome. (Table 1).
Win and Thandan (1996), reported the presence of flavonoids, glycosides, phenolic compounds, saponins, terpenoids, steroids and tannins in the rhizome of *C. longa.* The Petroleum ether extract of *C. longa* rhizome contain the presence of alkaloids, glycosides, tannins, flavonoids, steroids, saponins and terpenoids (Neeta *et al,* 2019). Saxena Tyoti *et al*. (2012) and Rajesh *et al*. (2013) isolated the phytochemicals such as steroids, glycosides, flavonoids, alkaloids, tannin and saponin from Methanolic extracts of *C. longa* rhizome.

Table 1: Phytochemical screening of Petroleum ether and Methanol extracts of *O. basilicum* leaf and *C. longa* rhizome

Bioactive Molecules	*O. basilicum*-*Petroleum Ether* extract	*O. basilicum*-Methanol extract	*C. longa*- Petroleum Ether extract	*C. longa*-Methanol extract
Alkaloids	-	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	+	+	-
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Phenols	+	+	+	+
Quinone	-	+	-	-
Phlobotannin	-	+	+	+

Antifeedant test

Percentage mortality rate of Insects in Petroleum Ether leaf extract of *O. basilicum* at 24, 48, and 72 hours

In Petroleum ether extracts of *O. basilicum* leaf 24, 48 and 72 Hours After Treatment (HAT), the mortality rate of *D. xanthomelas* was increased as the extract concentration increased. The mortality rate approximately increased from 3.24% to 5.00% at 5 µl concentration. At 10 µl concentration the mortality rate was increased from 3.66% to 5.66%. At 15 µl and 20 µl concentration the percentage of mortality rate was approximately 4.66% to 5.33%. Approximately the mortality rate in maximum dose of extract (25 µl) concentration were observed as 4.14% to 6.66% (Table 2).

Percentage mortality rate of Insects in Methanol extract of *O. basilicum* leaf at 24,48 and 72 hours

Percentage of mortality rate of *D. xanthomelas* after 24-, 48- and 72-hours treatment in methanol leaf extract of *O. basilicum* were observed approximately 2.33% to 7.00% at 5 µl concentration. At 10 µl concentration approximate percentage mortality was 2.32% to 6.33%. The percentage of mortality rate were observed 3.61% to 5.66% at 15 µl concentration. At 20 µl concentration the mortality rate percentage was observed as 4.66% to 5.66%. At 25 µl concentration the percentage mortality rate of insect was observed as 4.12% to 6.00% (Table 3).

Percentage mortality rate of Insects in Petroleum Ether rhizome extract of *C. longa* rhizome at 24,48 and 72 hours

After 24, 48 and 72 hours of *D. xanthomelas* treatment in petroleum ether extract of *C. longa* the mortality rate was observed to be 3.60% to 6.33% at 5 µl concentration. 4.30% to 6.33% at 10 µl concentration, 4.60% to 5.33% at 15 µl concentration. 4.30% to 5.60% at 20 µl concentration. 4.30% to 6.00% at 25 µl concentration (Table 4).

Percentage mortality rate of Insects in Methanol extract of *C. longa* rhizome at 24, 48 and 72 hours

The observed mortality percentage of *D. xanthomelas* at 5 µl, 10 µl, 15 µl, 20 µl and 25 µl concentration after 24-, 48- and 72-hours treatment was observed 2.30% to 6.66%, 3.33% to 6.33%, 3.00% to 6.11%. 3.66% to 6.15% and 3.33% to 6.33% respectively in methanol rhizome extract of *C. longa* (Table 5).

The study of Chander *et al*., (2000) also supported current findings of turmeric (*Curcuma longa*) extract has repellent action against *T. castaneum*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Sitophilus oryzae*, *Desonycha xanthomelae* and *Corcyra cephalonica* even after 3 months under laboratory conditions. The current study by Huag (2000) who tested two compounds extracted from *Curcuma longa* against *Sitophilus zeamais*, *D. xanthomelas* and *T. castaneum* for contact, fumigant and antifeedant activity.

Similar studies were reported (Smith, 1797), that 58.3% mortality were observed in army worm, *Spodoptera frugiperda* when acetonic solution of turmerone were mixed in artificial diet. An artificial diet treated with acetonic solutions of extracts of *C. Longa* rhizomes fed to the freshly emerged peach fruit flies, *Bactrocera zonata* for 16 days at 1,000, 500, and 250 ppm produced 84.7, 79.0, and 51.9% mortality, respectively.

Bhatnagar in 1993 reported that the essential oils and major constituents of aromatic plants, *O. basilicum* and *O. sanctum* were evaluated against several insects. The bioassay tests revealed that the essential oil of *O. basilicum* and its major constituent, methyl chavicol are more effective as compared to *O. sanctum*.

The studies conducted by Naveen *et al* (2021), evaluated the insecticidal properties of *O. basilicum* against cigarette beetle, *Lasioderma serricorne*. In contact toxicity test highest mean mortality was affected by 5% methanol extract (66.67%), followed by 4% methanol extract (58.33%), 3% methanol extract (52.22%), 2% methanol extract (47,22%) and 1% methanol extract (43.33%).

Table 2: Percentage mortality rate of Insects in Petroleum Ether leaf extract of *O. basilicum* at 24, 48, and 72 hours

Hours / Conc.	24 hrs	48 hrs	72 hrs
5 µl	3.24 ± 0.00^{a}	4.33 ± 0.33^{a}	5.00 ± 0.55 ^{e}
10 µl	3.66 ± 0.33 ^{b}	5.66 ± 0.33^{c}	5.66 ± 0.33 ^{c}
15 µl	4.66 ± 0.33 ^{a}	5.21± 0.55^{e}	5.33 ± 0.33 ^{a}
20 µl	4.66 ± 0.33 ^{a}	5.21± 0.00^{d}	5.33 ± 0.33 ^{a}
25 µl	4.14 ± 0.00 ^{c}	5.33 ± 0.33 ^{a}	6.66 ± 0.33 ^{b}

a-f means within a column followed by different letters are significantly, $P< 0.05$, Duncan multiple rank test

Table 3: Percentage mortality rate of Insects in Methanol extract of *O. basilicum* leaf at 24, 48 and 72 hours

Hours / Conc.	24 hrs.	48 hrs.	72 hrs.
5 µl	2.33 ± 0.33 ^{a}	4.66 ± 0.33 ^{c}	7.00± 0.66 ^{b}
10 µl	2.32 ± 0.33 ^{c}	5.33 ± 0.33 ^{d}	6.33 ± 0.66 ^{c}
15 µl	3.61 ± 0.33 ^{e}	5.65 ± 0.33 ^{a}	5.66 ± 0.33 ^{b}
20 µl	4.66± 0.33 ^{a}	4.66 ± 0.33 ^{e}	5.66 ± 0.33 ^{b}
25 µl	4.12 ± 0.00 ^{b}	5.00 ± 0.55 ^{c}	6.00 ±0.00 ^{b}

a-f means within a column followed by different letters are significantly, $P< 0.05$, Duncan multiple rank test

Table 4: Percentage mortality rate of Insects in Petroleum Ether rhizome extract of *C. longa* rhizome at 24,48 and 72 hours

Hours / Conc.	24 hrs.	48 hrs.	72 hrs.
5 µl	3.60 ± 0.33 [c]	5.00± 0.55 [e]	6.33 ± 0.66 [a]
10 µl	4.30 ± 0.33 [a]	2.61 ± 0.33 [e]	6.33 ± 0.33 [a]
15 µl	4.60 ± 0.33 [a]	4.31 ± 0.57 [b]	5.33 ± 0.33 [b]
20 µl	4.30 ± 0.33 [b]	5.00 ± 0.57 [c]	5.60 ± 0.88 [c]
25 µl	4.30 ± 0.33 [c]	4.66 ± 0.33 [c]	6.00 ± 0.00 [e]

a-f means within a column followed by different letters are significantly, *P*< 0.05, Duncan multiple rank test

Table 5: Percentage mortality rate of Insects in Methanol extract of *C. longa* rhizome at 24, 48 and 72 hours

Hours / Conc.	24 hrs.	48 hrs.	72 hrs.
5 µl	2.30 ± 0.33 [c]	4.66 ± 0.33 [d]	6.66 ± 0.66 [a]
10 µl	3.33 ± 0.33 [a]	5.33 ± 0.33 [a]	6.33 ± 0.33 [d]
15 µl	3.00 ± 0.00 [a]	6.00 ± 0.00 [a]	6.11 ± 0.00 [c]
20 µl	3.66 ± 0.33 [b]	5.33 ± 0.33 [b]	6.15± 0.00 [b]
25 µl	3.33 ± 0.33 [b]	5.33 ± 0.33 [b]	6.33± 0.33 [c]

a-f means within a column followed by different letters are significantly, *P*< 0.05, Duncan multiple rank test

Conclusion

The present study showed that Petroleum Ether and Methanol extract of *O. basilicum* leaf and *C. longa* rhizome is effective to control vegetable crop pest *D. xanthomelas*. The turmerone present in *C. longa* rhizome and the presence of major constituent methyl chavicol in *O. basilicum* extract shows insecticidal properties to control the insect's attack.

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