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Laboratory evaluation of chili and pepper oleoresins against certain sucking pests

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Abstract
The study investigated the efficacy of chili and black pepper oleoresins through dry film and contact toxicity bioassays against psyllids, mites, aphids, and mealy bugs under laboratory conditions. The oleoresins were found to possess high mortality rates at all concentrations tested. Among the insects tested, aphids were the most susceptible, with 80.33% mortality, even in the dry film bioassay's lowest concentration (0.1%). The decreasing order of efficacy was aphids > mites > psyllids > mealybugs. Against mealybugs, both the oleoresins were effective at 3 and 5% concentrations under contact toxicity bioassay only. Dry film bioassay was also found to be effective, indicating the presence of residual toxicity.

Keywords: chili and pepper oleoresins, sucking pests

Introduction
Over-reliance on chemical pesticides in crop pest management resulted in resistance and resurgence development, frequent secondary pest outbreaks, and toxic residue-laced end products (Fang *et al*., 2016) [6]. Due to the increasing concerns about environmental safety and the global demand for pesticide-residue-free food, the search for suitable alternatives is necessitated. With many plant species known to possess anti-insect properties and hundreds of insecticidal compounds extracted, botanical insecticides offer ample scope (Raghavendra *etal*., 2016). However, a few botanicals were commercially exploited: neem, rotenone, pyrethrum, and some essential oils (Isman, 2006) [5]. The main reasons for such a drawback are the difficulty getting plant biomass, standardization, and lack of formulations. Hence an attempt has been made to use spice oleoresins for pest management. Oleoresins are naturally occurring pungent oil and resin concentrations extracted from spices using organic solvents (llie *et al*., 2019) [7]. As they are concentrated spices, low active ingredient content and high potency enable their usage in small dosages like pesticides. Further, volatile and non-volatile components, better storage characteristics like thermal stability, low moisture, and fewer residues make them viable (Gudeva *et al.*, 2013) [10]. The study deals with the efficacy evaluation of red chili and black pepper oleoresins against major sucking pests.

Materials and Methods
Culturing of test insects
Sucking pests *viz.*, citrus psyllids (*Diaphorina citri* Kuwayama), red spider mites *(Tetranychus urticae* Koch), cotton aphids (*Aphis gossypii* Glover), and cotton mealybugs (*Phenacoccus solenopsis* Tinsley) were cultured in 25 days old citrus, bhendi, and cotton seedlings respectively. The seedlings were raised in cement pots (30 cm dia.), watered, and manured regularly. Field-collected insects were used to initiate the culture and were released onto the respective seedlings enclosed by mylar film cages covered on top using a cotton cloth. The cages were maintained parasitoid free. The insects were allowed to grow on the seedlings for a month, reintroduced to new plants, and held at 25+2°C and 65+5 % RH (Wakgari and Yigezu, 2018) [1].

Extraction of spice oleoresins and content estimation
The red chili fruits (Variety: Teja 4, M/S. Mahyco seed company, Mumbai) were collected from the farmers' fields at Sankarankovil, Tenkasi district, Tamil Nadu (9.4497°N lat. And 77.8360 °E long.). They were shade dried for three days, deseeded, and the pericarp was powdered. The powder was filled in a 40mm x 500mm long glass column and extracted using the solvent ethylene dichloride (1:10 ratio). The column was extracted for 1hr for the first wash, followed by five subsequent washes for 15 minutes each. The extracts were collected, filtered, and evaporated under reduced pressure in a boiling water bath. Then, the extractives were mixed with aqueous methanol at a 1:1 ratio in a magnetic stirrer (Remi, MS 500, Maharashtra) for 20 minutes. The solution was again poured into a column. The pure capsaicin oleoresin, which settled at the bottom, was collected, and the paprika at the top was discarded. Capsaicin oleoresin content was determined in a UV spectrophotometer (Pharmacia biotech, Ultrospec 2000, Sweden) by following the AOAC method (1996). Similarly, the black pepper oleoresin, piperine was extracted, and the content was determined.

Preparation of oleoresin concentrations
The extracted oleoresin containing 40% concentration was taken as stock. The concentrations *viz.*, 5.0, 3.0, 1.0, 0.7, 0.5, 0.3, and 0.1% were prepared by diluting 1250, 750, 250, 175, 125, 75, and 25 µl of the stock in emulsified water (10 ml).

Bio-efficacy studies
Contact toxicity bioassay
The respective host plant's fresh leaf with its petiole intact and covered with a wet cotton swab was placed separately in a Petri plate (80 mm dia.). Uniformly aged nymphs of

each test insect/mite @ 20 numbers were released per leaf. The Petri plate is lined internally with moist filter paper to avoid drying of the leaf. One ml of each oleoresin concentration was sprayed on the leaf with insects/mites using Potter's spray tower (Burkard manufacturing company Limited, England) at an xxx bar. The leaves were air-dried and maintained at 25 ± 1 °C, 80 ± 5% RH, and 16L: 8D photoperiod for 24 hours. Insects/mites were considered dead if they did not move when disturbed by a brush. Mortality was recorded 12 and 24 hours after treatment. Neem oil 0.5% treatment (Positive control) and an untreated control were maintained. The experiment was replicated three times and conducted as a Completely Randomized Design (CRD) (Khan *et al*., 2013; Tahmina *et al.*, 2020) [3, 11].

Residual toxicity bioassay

The inner sides of the Petri dishes (80 mm dia.) were coated separately with one ml of each oleoresin concentration by swirling it gently and air-drying at room temperature. Uniformly aged nymphs of each test insect/mite @ 20 numbers were released per Petri plate and maintained at 25 ± 1 °C, 80 ± 5% RH, and 16L: 8D photoperiod for 24 hours. Insects/mites were considered dead if they did not move when disturbed by a brush. Mortality was recorded 12 and 24 hours after treatment. Neem oil 0.5% treatment (Positive control) and an untreated control were maintained. The experiment was replicated three times and conducted as a Completely Randomized Design (CRD) (Paramasivam and Selvi, 2017) [2].

Statistical analysis

The percent mortality data were subjected to angular transformation where ever necessary and analyzed as suggested by Gomez and Gomez (1984) [12]. The mean values were compared using DMRT at a 5% significance level.

Result and Discussion

The results revealed the significant toxic effect of the oleoresins against sucking pests' nymphs at all the concentrations tested (5, 3, 1, 0.7, 0.5, 0.3, 0.1 & control). The effect was equally significant in both the contact and residual toxicity bioassays. Aphids were managed very effectively in residual toxicity bioassay at 0.1% concentration, followed by mites. However, against psyllids, both the oleoresins were found to impart comparatively less toxicity. Interestingly, at concentrations beyond 0.1% (0.3, 0.5, 0.7, & 1%), more than 90% mortality was imparted against mites and aphids. Further, at the highest concentrations tested (0.7 & 1%), a cent percent mortality was recorded against psyllids, mites, and aphids in both the bioassays. Both the oleoresins recorded more than 50% mortality in lower concentrations themselves. (Table 2).

However, against mealybugs, both the oleoresins imparted reduced mortalities. At the lower concentrations tested (0.5 & 0.7%), the oleoresins failed to kill any treated mealybugs. Even at 1% concentration, oleoresins could not impart any residual toxicity. In contact toxicity, only 36.33 and 34.33% mortalities were recorded in chili and pepper oleoresins, respectively. Further, at the highest concentration tested (5%), residual toxicity recorded was 30 and 31.66% in chili and pepper oleoresins, respectively (Table 3). This might be due to the mealy coating in the insects.

The decreasing order of efficacy was aphids > mites > psyllids > mealybugs. The present findings were in correlation with Li *et al*. 2019 [13]. They reported capsaicinoids were effective against *Aphis gossypii* with 152.82 mgL^{-1} as LC_{50}. Similarly, Hasan and AI-Jayashi (2021) [15] similarly reported hot pepper extract's superiority in controlling cotton aphids. Iamba and Waiviro (2021) [14] reported chili extract's effect in lowering diamondback moth.

The results confirmed that oleoresins are a potential source for the management of sucking pests, and their effectiveness could be improved through formulations.

Table 1: Spice oleoresins' toxicity against sucking pests under laboratory condition

Conc.	Percent mortality*											
	Chili oleoresin						Pepper oleoresin					
	Contact toxicity bioassay			Residual toxicity bioassay			Contact toxicity bioassay			Residual toxicity bioassay		
	Psyllids	Mites	Aphids	Psyllids	Mites	Aphids	Psyllids	Mites	Aphids	Psyllids	Mites	Aphids
1%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
0.7%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
0.5%	80.00 (63.52)	99.50 (86.71)	100 (90.00)	75.00 (60.05)	98.33 (82.56)	100 (90.00)	78.00 (62.09)	97.50 (80.90)	100 (90.00)	73.00 (58.73)	96.33 (78.93)	100 (90.00)
0.3%	56.66 (48.84)	91.66 (73.22)	99.66 (88.07)	52.50 (46.43)	90.00 (71.53)	99.66 (88.07)	54.66 (47.68)	89.66 (71.24)	97.66 (81.22)	50.50 (45.28)	88.00 (69.76)	97.66 (81.22)
0.1% Neem oil @ 0.5%	43.33 (41.15) 34.33 (35.85)	70.00 (56.76) 63.00 (52.88)	83.00 (65.62) 58.00 (49.48)	38.33 (38.23) 26.66 (30.86)	66.00 (54.31) 52.00 (46.30)	82.33 (65.15) 50.00 (44.98)	41.33 (39.99) 34.00 (35.67)	68.00 (55.52) 62.00 (51.77)	81.00 (64.13) 60.00 (50.74)	36.33 (37.05) 22.66 (28.86)	64.00 (53.11) 50.00 (44.98)	80.33 (63.67) 49.66 (44.52)
Control	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
CD 5%	4.11	2.43	2.49	4.45	0.60	2.76	4.04	1.19	0.91	4.41	0.49	1.46
SE(m)	1.32	0.78	0.80	1.43	0.19	0.88	1.29	0.38	0.29	1.41	0.15	0.47

*Values mean of three replications

Values in parentheses are arc sine transformed

Table 2: Spice oleoresins' toxicity against Cotton mealy bug, *Phenacoccus solenopsis* under laboratory condition

Treatments	Percent mortality*			
	Chili oleoresin		Pepper oleoresin	
	Contact toxicity bioassay	Residual toxicity bioassay	Contact toxicity bioassay	Residual toxicity bioassay
5%	85.83 (67.92)	31.66 (34.21)	83.83 (66.31)	30.00 (33.19)
3%	66.50 (54.61)	20.00 (26.55)	64.50 (53.40)	18.00 (25.04)
1%	36.33 (37.05)	0 (0.00)	34.33 (35.85)	0 (0.00)
0.7%	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
0.5%	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Neem oil @ 0.5%	20.00 (26.55)	18.00 (26.00)	20.00 (26.55)	20.00 (26.55)
Control	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
CD 5%	1.71	0.26	1.62	0.26
SE(m)	0.54	0.08	0.52	0.08

*Values mean of three replications

Values in parentheses are arc sine transformed

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