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Bioefficacy of *Tagetes minuta* L. against *Aphis craccivora* Koch (Hemiptera, Aphididae)

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ABSTRACT: Efficacy of solvent fractions of *Tagetes minuta* L. (hexane, chloroform and aqueous fractions of aerial parts) was evaluated against *Aphis craccivora* Koch at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2%) in the laboratory. At higher concentrations, all the fractions recorded significant reduction in aphid count; maximum reduction after 120h of treatment was in hexane fraction at 0.2 per cent (90.00%), followed by chloroform fraction at 0.2 per cent (86.67%) and aqueous fraction at 0.2 per cent (85.00%). The best concentrations of the three solvent fractions of *T. minuta* identified in the laboratory were evaluated against the aphid on cowpea, in a pot culture experiment. By 10^{th} day of treatment, all the fractions at 2 per cent concentration reduced population of aphids, with the highest reduction in hexane fraction. © 2023 Association for Advancement of Entomology

KEYWORDS: Mexican marigold, solvent fractions, hexane fraction, aphid management

INTRODUCTION

The cowpea aphid, *Aphis craccivora* Koch (Hemiptera, Aphididae) is an important sucking pest of leguminous crops throughout India (Jagdish *et al.*, 2011). It is a serious pest of cowpea, feeding on aerial parts of the plant and leading to significant yield loss (Veeranna and Adivappar *et al.,* 2019). It also causes indirect damage to the plant by excreting copious amount of honey dew, which leads to the development of sooty mould on the surface of leaves, thereby reducing the rate of photosynthesis; besides, it is also a vector of important viral diseases affecting the legumes (Ghosh *et al*., 2017). Synthetic insecticides are being extensively used for aphid management in vegetable crops in India. The popular chemical insecticides used against aphids include neonicotinoids like imidacloprid, acetamiprid and thiamethoxam. The excessive and indiscriminate use of synthetic pesticides for their management has, however, led to the development of resistance in the populations of aphids. *A. craccivora* has been reported to exhibit considerable degree of resistance to newer insecticides like imidacloprid (Dawood and Farghaly, 2016) and dinotefuran (Mokbel and Mohamed, 2009), among many others. Hence, there is a need for identifying alternative, environmentally benign, effective, and biodegradable pesticides with greater selectivity against the aphid pest. In this

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context, botanical pesticides are ideal alternatives for synthetic pesticides due to their rapid degradation, target specific nature and less phytotoxicity (Vasquez *et al*., 2016). *Tagetes minuta* L., commonly known as Mexican marigold is an annual herb that belongs to the family, Asteraceae. The biological activities of the plant have been documented worldwide. Insecticidal effects of *T. minuta* have been studied against several pests of field crops, stored products and public health (Perich *et al*., 1995; Shahzadi *et al*., 2010; Phoofolo *et al.,* 2013). Few studies have been conducted in India, particularly in Kerala, to evaluate the insecticidal effects of this plant; hence, a study was conducted to identify the insecticidal effects of the solvent fractions of *T. minuta* on *A. craccivora*.

MATERIALS AND METHODS

Adults of *A. craccivora* collected from the culture maintained, in the polyhouse of Department of Agricultural Entomology, College of Agriculture, Vellanikkara, Kerala, India were used to initiate the laboratory culture. The aphids were allowed to infest cowpea seedlings grown in pro trays maintained in rearing cages in the laboratory. New seedlings were allowed to be infested periodically to maintain the culture. In order to obtain uniform aged adult aphids for the study, a few seedlings from the culture were selected and all adults were removed, retaining the nymphs. On the next day, the freshly moulted adults were collected from those seedlings and used for the study.

Preparation of solvent fractions: *T. minuta* plants, cultivated in the pots, in the Department of Floriculture and Landscaping, College of Agriculture, Vellanikkara, Kerala, India were used for the study. The above-ground parts of the plant were harvested at the flowering stage, shade dried and pulverized. The botanical was then extracted sequentially, based on the polarity of the solvents into three separate fractions, using hexane (non-polar), chloroform (medium polar) and water (highly polar). The aerial parts of the plant were dried and pulverized material was weighed (100g each conical flask) and extracted using hexane (3 times of volume) by placing in a rotary shaker for 48h. The solution was then filtered and the filtrate was concentrated by a rotary evaporator at 40ºC, to obtain the hexane fraction. The residue obtained during filtration was re-extracted sequentially using chloroform, followed by water, following the same procedure as hexane extraction, to obtain chloroform fraction and aqueous fraction, respectively (Auamcharoen and Chandrapatya, 2015). The three solvent fractions were used for bioefficacy studies against *A. craccivora.*

Laboratory bioassay: The hexane, chloroform and aqueous fractions of *T. minuta* were evaluated at five different concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 %) for efficacy against *A. craccivora*. The treatment concentrations were prepared by dissolving the required quantity of each fraction in distilled water along with the emulsifier, Triton X-100 (20µl 50ml^{-1}). Cowpea seeds were sown in individual paper cups (one seed per cup) filled with potting mixture (soil and vermicompost in 1:1 ratio) and the seeds were allowed to sprout and grow. At four leaf stage, adult aphids from the laboratory culture were released using a camel hair brush at the rate of 20 per plant and allowed to settle. The seedlings were then sprayed with appropriate treatments using a hand atomizer (3ml per seedling). Three replications were maintained for each concentration of the fractions. Seedlings sprayed with water plus emulsifier, served as control. An absolute control was also maintained without any treatment. The experiment was laid out in Completely Randomized Design (CRD) with 17 treatments and three replications. Nymphs produced viviparously if any, were removed from the experimental seedlings periodically, to avoid any experimental error due to population build up. Number of adult aphids on the plants was recorded at 24, 48, 72, 96 and 120h of spraying and reduction in aphid count was calculated. Data on reduction of aphid was subjected to analysis of variance using the software, GRAPES 1.0.0, developed by Kerala Agricultural University.

Evaluation of fraction in pot culture: A pot culture experiment was carried out to evaluate the efficacy of the most effective concentration of each fraction of *T. minuta* against *A. craccivora* in cowpea, during July - September, 2022. The best concentration each of hexane (0.2%), chloroform (0.2%) and aqueous (0.2%) fractions were selected for evaluation based on their efficacy in the laboratory study against the adult aphids. The efficacy of the selected fractions was compared with those of neem oil emulsion (2%), azadirachtin (1% EC, 3ml L^{-1}) and horticultural mineral oil (2%). An untreated control was also maintained. The experiment was laid out in CRD with seven treatments, each replicated thrice with eight plants per replication.

The seeds of cowpea variety - Bhagyalakshmi (bush type) were sown in individual polybag (35cm x 20cm x 20cm) filled with potting mixture (soil, coir pith compost and cow dung in 2:1:1 ratio). All cultural practices were carried out as per Package of Practices Recommendations, KAU (2016). Aphids were allowed to infest the 20-day-old cowpea plants by keeping aphid infested cowpea seedlings in paper cups, at the base of each plant in polybag. Treatments were imposed 20 days after the release of aphids, using a hand sprayer. For recording the aphid population in treatments, three plants were selected randomly from each treatment replication. Number of aphids (both nymphs and adults) was recorded from three shoot bits of 5cm length per plant, excised from three tender shoots (Bindu, 1997). Pre-treatment count one day prior to treatment and post treatment counts of aphids at 1, 3, 7 and 10 days after treatment (DAT) application were counted by dislodging the aphids from each shoot bit, on to a white paper.

The data on mean number of aphids (per 5cm shoot length) before and after treatments were subjected to analysis of covariance (ANCOVA) using the software, GRAPES 1.0.0. In order to accommodate the variations in pre-count, the transformed data were analysed by taking population counts prior to the first application as covariate and ANCOVA was done for observations at 1, 3, 7 and 10 DAT. The result obtained was subjected to LSD (Least Significance Difference Test). The mean per cent reduction in population was also worked out 10 DAT.

Qualitative phytochemical analysis: Qualitative analysis of hexane (nonpolar) and chloroform (medium polar) fractions of *T. minuta*, was performed to determine the predominant phytochemical constituents in the fractions. However, aqueous (polar) fraction was not analysed as ample literature on the constitution of polar fractions of *T. minuta* is available. The hexane and chloroform fractions were subjected to GC-MS/MS analysis at Sophisticated Analytical Instrumentation Facility (SAIF), IIT, Mumbai and the major compounds present in the fractions were recorded.

RESULTS AND DISCUSION

Laboratory evaluation of solvent fractions: There was a sudden decline in aphid count on treated seedlings up to 48h of treatment, after which the population reduction was less pronounced. All three solvent fractions recorded the maximum reduction in aphid count at the highest concentration (0.2%) tested. Within 24h of treatment the hexane fraction (@0.2%) recorded 61.67 per cent reduction, followed by chloroform (60%) and aqueous fractions (58.33%), which were on par with each other. At lower concentration (0.15%), hexane fraction and aqueous fraction also reduced aphid count (60 and 50% respectively) and were on par with the above treatments. After 48h, hexane fraction (@0.2%) resulted in as high as 83.33 per cent reduction. Chloroform fraction (0.2%) also showed significant reduction (78.33%), on par the hexane fraction. Other concentrations of solvent fractions also showed reduction in aphid count at 48h. From 48 to 120h of treatment, the reduction in aphid count was less prominent in all the treatments. After 120 h of treatment, there were significantly higher reduction in aphid count in hexane fraction (@0.2%), hexane fraction (@0.15%), chloroform fraction (@0.2%), chloroform fraction (@0.2%) and aqueous fraction (@0.2%) (recording 90.00, 86.67, 86.67, 85.00 and 85.00% respectively) and were on par with one another (Table 1).

Evaluation of fractions in pot culture: All the treatments reduced the aphid population considerably 10 DAT. Among the fractions, hexane fraction recorded the lowest population (11.18 aphids per 5cm of shoot) resulting in 78.95 per cent reduction in aphid population. The hexane fraction

Table 1. Laboratory evaluation on the bioefficacy of *Tagetes minuta* solvent fractions against *Aphis craccivora*

Treatment	Reduction in aphid count (%) – hours after treatment				
	24h	48h	72h	96h	120h
Hexane fraction 0.025 %	23.33^{fg} (28.67)	40.00^{hi} (39.21)	53.33^{gh} (46.92)	56.67^{gh} (48.84)	56.67^{f} (48.84)
Hexane fraction 0.05 %	40.00^{cde} (39.21)	53.33^{fg} (46.94)	60.00^{fg} (50.85)	65.00^{fg} (53.76)	65.00^{de} (53.76)
Hexane fraction 0.1%	43.33^{cd} (41.13)	63.33^{e} (52.78)	68.33^{def} (55.82)	75.00^{de} (60.07)	76.67^{bc} (61.15)
Hexane fraction 0.15 %	60.00^{ab} (50.79)	75.00^{b} (60.07)	76.67^{bcd} (61.15)	80.00^{cd} (63.55)	86.67^{a} (68.66)
Hexane fraction 0.2 %	61.67^{a} (51.81)	83.33^{a} (65.95)	86.67^{a} (68.66)	88.33^{a} (70.11)	90.00^{a} (71.57)
Chloroform fraction 0.025 %	30.00^{ef} (33.16)	48.33^{gh} (44.04)	55.00^{gh} (47.88)	56.67^{gh} (48.84)	58.33^{ef} (49.80)
Chloroform fraction 0.05 %	36.67^{de} (37.20)	65.00^{de} (53.76)	70.00^{de} (56.84)	71.67^{ef} (57.86)	73.33^{bc} (59.00)
Chloroform fraction 0.1 %	38.33^{cde} (38.19)	66.67^{cde} (54.75)	71.67^{cde} (57.86)	75.00^{de} (60.00)	76.67^{bc} (61.15)
Chloroform fraction 0.15 %	48.33^{bcd} (44.01)	73.33^{bc} (58.93)	80.00^{abc} (63.55)	81.67^{bcd} (64.81)	85.00^{a} (67.40)
Chloroform fraction 0.2 %	60.00^{ab} (50.79)	78.33^{ab} (62.29)	85.00^{a} (67.40)	86.67^{ab} (68.66)	86.67^{a} (68.66)
Aqueous fraction 0.025%	15.00^{gh} (22.29)	36.67^{I} (37.26)	45.00^{h} (42.12)	46.67^{I} (43.09)	48.33^{g} (44.04)
Aqueous fraction 0.05%	28.33^{ef} (32.14)	45.00^{ghi} (42.12)	53.33^{gh} (46.92)	55.00^{hi} (47.91)	58.33^{ef} (49.83)
Aqueous fraction 0.1%	38.33^{cde} (38.22)	61.67^{ef} (51.76)	65.00^{ef} (53.76)	70.00^{ef} (56.79)	70.00^{cd} (56.79)
Aqueous fraction 0.15%	50.00^{abc} (45.00)	63.33^{e} (52.74)	71.67^{cde} (57.86)	75.00^{de} (60.07)	78.33^{b} (62.29)
Aqueous fraction 0.2 %	58.33^{ab} (49.80)	71.67^{bcd} (57.86)	81.67^{ab} (65.00)	83.33^{abc} (66.15)	85.00^{a} (67.40)
Control (Water + emulsifier)	13.33^{h} (21.34)	13.33^{j} (21.34)	16.67^{I} (24.05)	18.33^{j} (25.31)	18.33^{h} (25.31)
Absolute control	1.67^{i} (4.31)	10.00^{j} (18.43)	11.67^{I} (19.89)	13.33^{j} (21.34)	13.33^{h} (21.34)
LSD (0.05)	7.329	4.881	5.789	4.974	4.568

Figures in parentheses are arc sine transformed values; Means followed by common letter(s) do not significantly differ at P=0.05%

was on par with the neem oil emulsion (7.57 aphids/ 5cm shoot) that reduced aphid population by 87.59 per cent. The chloroform and aqueous fractions were on par with each other, reducing aphid population by 72.43 and 68.20 per cent, respectively. Aqueous fraction recorded aphid population (23.11) on par with azadirachtin (25.74), whereas, chloroform fraction was superior to azadirachtin recording a lower population of 18.41 aphids per 5 cm of shoot (Table 2).

Phytochemical constituents in solvent fractions: Qualitative phytochemical analysis of the bio-active fractions *viz.*, hexane and chloroform fractions GC-MS/MS identified the presence of 4á-phorbol 12,13-didecanoate, 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in hexane fraction and 4-o-methylphorbol 12, 13- didecanoate and milbemycin b in the chloroform fraction.

In the laboratory, on treatment application, the aphids moved away from the treated seedlings, suggesting a strong repellent and/or antifeedant action by the solvent fractions of *T. minuta*. In the pot culture study, the solvent fractions could significantly reduce the aphid population on cowpea. The efficacy of the hexane fraction of *T. minuta* was found comparable to neem oil while that of chloroform and aqueous fractions was comparable to azadirachtin 1 EC, which are widely recommended in the management of aphids in various crops (Sarvaiya *et al.*, 2018).

Volatile constituents present in several botanicals are known to trigger sudden repellent action in the aphids (Dardouri *et al.*, 2019). Volatile compounds such as D-limonene, dihydro-tagetone, (E)-tagetone, (Z)-tagetone, (Z)-beta-ocimene and allo-ocimene were reported to be present in *T. minuta* and are known to have repellent properties against insects (Kimutai *et al.*, 2015). Polar fractions of *T. minuta*, extracted using polar solvents such as methanol and water were reported to be composed

Table 2. Effect of *Tagetes minuta* solvent fractions on *Aphis craccivora* in cowpea pot culture

Treatment	Mean live aphids/ 5cm shoot length at - DAT					Reduction (%)
	PTC*	1	3	7	10	
Hexane fraction 0.2 %	53.11 (7.28)	20.37[c] (4.51)	15.29[d] (3.91)	12.59[c] (3.54)	11.18[d] (3.33)	78.95
Chloroform fraction 0.2%	66.78 (8.17)	27.33[b] (5.22)	23.33[bc] (4.81)	19.69[b] (4.39)	18.41[c] (4.27)	72.43
Aqueous fraction 0.2%	72.67 (8.52)	30.04[b] (5.48)	20.74[c] (4.55)	18.63[b] (4.31)	23.11[bc] (4.81)	68.20
Neem oil emulsion 2%	61.18 (7.82)	11.89[d] (3.45)	9.96[e] (3.16)	7.93[d] (2.81)	7.59[de] (2.75)	87.59
Azadirachtin 1% EC (3 ml L^{-1})	80.37 (8.96)	29.00[b] (5.38)	25.85[b] (5.08)	24.63[b] (4.95)	25.74[b] (5.07)	67.97
Horticultural mineral oil 2%	33.93 (5.82)	8.45[e] (2.90)	6.48[f] (2.55)	6.38[d] (2.52)	5.59[e] (2.36)	83.52
Untreated control	43.74 (6.61)	50.41[a] (7.09)	44.36[a] (6.66)	51.22[a] (7.15)	61.78[a] (7.85)	–
LSD (0.05)	NS	0.413	0.478	0.715	0.590	–

*PTC=Pre-treatment count; #Reduction at10 DAT (%); Figures in parentheses are square root transformed values; Means followed by common letter (s) do not significantly differ at P=0.05 %

of various carbohydrates, proteins, phenols, flavonoids, terpenoids and alkaloids (Rikisahedew, 2018). The terpenoid, D-limonene present in the polar fraction of *T. minuta* is known to possess significant insecticidal properties (Karr and Coats, 1987).

Qualitative phytochemical analysis of hexane and chloroform fractions in the present study showed phorbol esters as predominant compounds. The antifeedant properties of the phorbol esters against some insect pests have already been documented. Phorbol esters extracted from *Jatropha* oil were evaluated for insecticidal activity against the third instar larvae of *Spodoptera frugiperda* (JE Smith). At highest concentration of the phorbol esters (0.25 mg ml^{-1} w/v), food consumption and relative growth rate of *S. frugiperda* larvae reduced (by 33 and 42% respectively), apart from exhibiting contact toxicity (Devappa *et al*., 2012). Phorbol esters are also known to affect signal transduction pathways by acting on the biological membranes (Goel *et al.*, 2007). Among the two phorbol esters identified, the role of 4á-phorbol 12, 13-didecanoate as a prominent TRPV (Transient receptor potential cation channel) agonist has been widely documented (Alexander *et al*., 2012). Similarly, milbemycin b, identified in both hexane and chloroform fractions, is a GABA gated chloride channel agonist (Ozoe, 2012), like the avermectin group of insecticides. The insecticidal activities of this compound have been widely documented against several insects (Bobade, 2019).

There are also reports on the detrimental effects of volatile compounds present in *T. minuta viz.,* â-caryophyllene, limonene and (Z) ocimene on the reproduction of aphids. Tomova *et al.* (2005) reported up to 100 per cent reduction in reproduction in three species of aphids, *viz., Acyrthosiphon pisum*, *Myzus persicae* and *Aulacorthum solani*, on exposure to these compounds. The efficacy of the *T. minuta* fractions against *A. craccivora* in the present study could be the combined effect of repellency, antifeedancy, and neurotoxicity and reproduction inhibition. Though insecticidal effects of *T. minuta* against different species of aphids have been documented from different parts of the world, most of the studies evaluated the crude aqueous extracts of the botanical in the field. Limited studies were conducted to evaluate the solvent fractions of *T. minuta.* When Ali *et al*. (2019) evaluated the bio-efficacy of aqueous leaf extracts of *T. minuta* along with three other botanicals *viz., Calotropis procera, Argemone mexicana* and *Azadirachta indica* against the mustard aphid, *Lipaphis erysimi* on Indian mustard, *Brassica juncea*, at higher concentration (1: 2.5 g ml^{-1}), *T. minuta* reduced *L. erysimi* population by 96.38 per cent. Kora and Teshome (2016) evaluated the aqueous extracts of chilli, garlic, ginger and *T. minuta* for their insecticidal property against green pea aphid, *Acrythosiphon pisum* in the field. The aphid population was reduced to zero in the plants treated with *T. minuta*, chilli and ginger. The above studies show that *T. minuta* extracts can reduce aphid population build up considerably, in the field. Phoofolo *et al*. (2013) had evaluated the aphidicidal activities of the crude extracts *T. minuta* against the cabbage aphid, *Brevicoryne brassicae*. A comparison was made on lethal and sub-lethal effects of the crude extracts from acetone, methanol, water and a mixture of acetone/methanol/ water (7:7:1 v:v). The mixture produced the most toxic extract, followed by methanol and water; whereas acetone extract was the least toxic. The study also demonstrated that the crude extract of *T. minuta* obtained using water as a solvent is as effective as crude extracts from organic solvent systems in terms of efficacy against cabbage aphids. This observation corresponds to the results of the present study where all the three solvent fractions of *T. minuta* (hexane, chloroform and water) were effective in reducing the aphid population.

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