Evaluation of different plant extracts in the management of tukra disease in mulberry caused by pinkmealybug (*Maconellicoccushirsutus*green)

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Abstract

Tukra is one of the major problems in mulberry garden caused by pink mealybug *Maconellicoccushirsutus* (Green), which affects the mulberry yield. In the present study, five different concentrations [10%, 30%, 50%, 70% 90% and 100% (crude extract)] of two herbal extracts (*Ocimum sanctum* and *Pongamia pinnata)* were tested against egg hatchability, mortality of nymphs and adults and fecundity of mealybug. Among these two botanical extracts, the maximum decrease in egg hatchability, nymph and adult mortality (100 per cent) and fecundity (2.67%) were observed in *P. pinnata* extract followed by *O.* sanctum (5.34%) treated groups.

Key words: *Bombyx mori, Ocimum sanctum, Pongamia pinnata, mulberry, tukra.*

INTRODUCTION

The production of raw silk, which is the only food used to raise silkworms, is mostly determined by mulberry farming [1]. Numerous factors, including diseases (24%), insect pests (18%), weeds (7%), and others (51%), have an impact on the yield of mulberry leaves [2]. There have been reports of over 300 different insect and non-insect pest species infesting mulberry crops, resulting in both qualitative and quantitative harm [3].

Mealybugs are a dangerous and invasive type of pest that feed on sap and are classified as polyphagous. It is commonplace on a global scale. One of the main pests of mulberries is the pink mealy bug, M. hirsutus, which causes significant damage and recurrent losses in leaf

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yield of roughly 3000-6000 kg /ha -1 /year [4]. The insect produces the distinctive illness "tukra" in mulberry gardens that are rain-fed or irrigated. The crumpling's folds and tangles are home to the pest [5]. Clusters of immature and mature mealy bugs are found beneath the overlapping leaf sheath on the stalks, below the node, and extending upwards and downwards to the other internodes and buds [6]. The impacted tukra plant's morphological modifications include a stop to the stem's linear growth, thickening of the petiole, and a reduction in the internodal distance, which results in a distinctive bunchy top. Mealy bugs use piercing stylets to feed on the sap of plant cells [7]. By emptying the sap from the plant, this penetrating mechanical feeding harms it and may spread bacterial and fungal diseases. Low insect levels rarely cause substantial damage, while severe infestations have the potential to destroy a plant. The manufacturing of silkworm cocoons performs poorly when leaf quality declines [8].

Because of their sheltered habitat and white waxy or mealy coating, pink mealy bugs are difficult to kill [9]. In general, it is not recommended to use insecticides when raising mulberries [10]. Synthetic pesticides pose a risk to human health and the environment because of their high toxicity to non-pest creatures and the potential to create pest resistance when used repeatedly [11].

In mulberry gardens, non-chemical methods such as using botanicals have shown to be an effective substitute for pesticides in recent times. Botanical insecticides have little toxicity to non-target organisms and the ecosystem[13] and are biodegradable [12, 13]. Numerous plants have yielded hundreds of phytopesticidal chemicals. Several plants have been identified as common insecticidal plants that can be used to control pest population in mulberry gardens, including Lantana camara L. and Catharanthus roseus L. [14], as well as Neem, Pongamia, Adathoda, Chrysanthemum, Turmaric, Onion, Garlic, Tobacco, Ocimum, Custard apple, Zinger, and some other plants 15, 16, 17, and 18. They function as insect growth regulators (IGR), repellents, feeding deterrents, and confusants, among other methods [18,19, 20]. Compared to extracts made from stems, fruits, flowers, and leaves, Balanites aegyptiaca root extracts exhibit greater activity against Maconellicoccus hirsutus [21]. Considering this, the current investigation was carried out to determine the impact of two medicinal plant extracts, Occimum sanctum Linn. (Laminaceae) and Pongamia pinnata Linn. (Fabaceae), on the various stages of the pink mealy bug M. hirsutusegg, including hatchability, nymph and adult mortality, and fecundity.

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MATERIALS AND METHODS

Mulberry plant (MR_2 variety) was maintained by standard agronomic practices.Few mulberry plants infested with mealybug were randomly selected and the population of pink mealy bug (*M. hirsutus*) at different stages (egg, nymph and adults) was counted and expressed as number per leaf. Observation was made just before spraying.

Collection of medicinal plants

The leaves of tulasi*(Occimum sanctum L.)* andPongam*(Pongamia pinnata L.)* were selected and used as study materials.These plants were abundantly available in the study area.

Preparation of aqueous extracts

The gathered leaves were separately shade-dried at room temperature and cleaned with distilled water in order to prepare an aqueous extract.200 ml of boiling water was used to soak 25 g of the weighed plant leaves that had been powdered. Using a conical flask, the mixture was boiled for thirty minutes and then left for twenty-four hours. Whatman filter paper No. 1 was used to filter the extract. Different concentrations of botanicals (10, 30, 50, 70, 90, and 100% (crude extract)) were made using distilled water, and the extract was kept at 40C until needed.

Experimental design

Tukra infested mulberry leaves at early stage were identified and different concentrations of these extracts were sprayed (using hand sprayer)on different stages of mealybugs (eggs, nymph and adults) simultaneously both on dorsal and ventral surface of mulberry leaves under in-vitro condition. To prevent contamination, great care was taken to completely wash the hand sprayer with water before using another herb. Totally there were 6 treatments in each plant extracts with five replications. The untreated group serves as control. The percentage of egg hatchability, mortality counts of both nymphs and adults, and fecundity of adults were recorded daily.

Results

The result shows that the hatching of eggs delayed by 4 to 8 days when *M. hirsutus* eggs were treated with *O. sanctum* (Table 1.1). The maximum decrease in percentage of

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hatchability was observed at 100% *O. sanctum* extract (4.06%). Effect of *P. pinata* extract on the hatchability of *M. hirsutus* eggs was shown in Table 1. 2. The hatchability of eggs was delayed by 5 to 8 days and extended up to 12 days, when the eggs were treated with six different concentrations of *P. pinata* aqueous leaf extract. At higher concentration there was complete inhibition of hatching. Maximum suppression of hatching was recorded in 100% concentration (3.23%).

With *O. sanctum* (100% concentration) 100% mortality of nymph was observed on 5^{th} day, on the other hand 100 % mortality was observed on 15^{th} day at 10 % concentration (Table 1.3). Table 1.4 indicates that with 100 % *P. pinnata* extract 100 % mortality was recorded on 4^{th} day, when compared to control (99.14%).Table1. 5 shows the effect of *O. sanctum* extract on the mortality of adult *M. hirsutus* bugs. Among the different concentrations of botanical extracts at 100%. 100 per cent mortality of adult was observed on the 9^{th} day, whereas in case of 90%, 70%, 50%, 30% and 10% the mortality was on 10^{th}, 11^{th}, 12^{th}, 13^{th} and 14^{th} days respectively. The concentrations of *P. pinata* extract showed significant effect on the mortality of adult bugs (Table1.6). Among the different concentrations of botanical extracts, 100 per cent mortality of adults was observed on the 7^{th} day (at 100% concentration), whereas in case of 90%, 70%, 50%, 30% and 10% mortality was on 14^{th}, $12^{th,}$, 11^{th}, 10^{th} and 9^{th} days respectively. The mortality of adults was on par with the control in all the extracts treated at lower concentrations.

Table 1.7 indicates the effect of different concentrations of medicinal plant extracts on the fecundity of *M. hirsutus*. Minimum fecundity (13 eggs) was observed in *P. pinata* extract and maximum fecundity (486 eggs) was observed in control, as well as at lowest concentration of treated groups.

Table 1.1. Effect of *O. sanctum* extract on the hatchability of *M. hirsutus* eggs:

Concentration (%)	Total number of eggs treated	Eggs hatched/ day												Total number of eggs hatched	Percentage of hatchability (%)
		1	2	3	4	5	6	7	8	9	10	11	12		
Control	350	-	-	-	135	120	50	39	3	-	-	-	-	347±32.16	99.14
10	275	-	-	-	-	8	14	10	40	89	60	15	5	241	87.63

														±24.28	
30	325	-	-	-	-	-	9	20	50	102	40	10	7	238±28.13	73.23
50	357	-	-	-	-	-	-	-	46	60	50	20	15	191 ±18.29	53.50
70	329	-	-	-	-	-	-	30	7	20	3	-	-	60 ± 11.54	18.23
90	295	-	-	-	-	-	-	-	7	5	10	11	-	33 ± 6.73	11.18
100	344	-	-	-	-	-	-	-	-	6	3	5	-	14 ± 4.13	4.06

Table 1.2. Effect of *P. pinnata* extract on the hatchability of *M. hirsutus* eggs:

Concentration (%)	**Total number of eggs treated**	**Eggs hatched/ day**												**Total number of eggs hatched**	**Percentage of hatchability (%)**
		1	2	3	4	5	6	7	8	9	10	11	12		
Control	350	-	-	-	135	120	50	39	3	-	-	-	-	347±32.16	99.14
10	329	-	-	-	-	-	42	93	60	32	26	17	-	270 ± 27.34	82.06
30	278	-	-	-	-	-	51	102	63	28	12	6	4	266±226.75	70.37
50	373	-	-	-	-	-	-	39	63	14	13	9	-	138 ± 18.54	50.54
70	316	-	-	-	-	-	-	21	14	8	6	4	-	53 ± 12.87	16.77
90	342	-	-	-	-	-	-	-	16	6	4	2	-	28 ± 9.60	8.18
100	278	-	-	-	-	-	-	-	-	3	2	4	-	9± 3.24	3.23

Table 1.3. Effect of *O. sanctum* extract on the mortality of *M. hirsutus*at nymphal stage.

Con cen trat ions (%)	**Mortality (%)**																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Con trol	-	-	-	15.0 ±	15.4 ±	23.6 ±	32.6	45.4 ±	45.4 ±	45.4 ±	45.4 ±	45.4	45.4 ±	-	-	-	-	-

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				3.16	3.50	4.15	± 5.3	2.96	2.96	2.96	2.96	± 2.96	2.96					
10	6.2 ± 2.58	14.00 ± 2.91	24.4 ± 3.36	35.8 ± 3.56	41.00 ± 3.16	50.4 ± 2.70	56.6 ± 2.70	62.4 ± 3.04	75.00 ± 2.23	81.2 ± 1.92	85.2 ± 2.53	90.8 ± 2.77	94.4 ± 2.07	97.8 ± 1.92	100.0 ± 0.00	-	-	-
30	15.2 ± 3.56	19.00 ± 2.23	25.4 ± 2.07	33.6 ± 4.03	42.00 ± 2.91	53.00 ± 3.87	61.6 ± 3.04	74.4 ± 3.04	80.8 ± 2.77	85.6 ± 2.30	88.8 ± 2.38	93.2 ± 1.92	100.0 ± 0.00	-	-	-	-	-
50	34.4 ± 3.36	40.4 ± 3.36	45.6 ± 2.30	51.6 ± 3.04	58.4 ± 2.30	71.4 ± 3.97	77.2 ± 2.58	86.2 ± 2.86	93.4 ± 2.40	97.00 ± 1.58	100.0 ± 0.00	-	-	-	-	-	-	-
70	45.6 ± 2.88	56.4 ± 2.07	62.00 ± 2.54	67.00 ± 1.58	74.4 ± 2.30	75.00 ± 3.87	83.4 ± 2.40	95.6 ± 3.64	98.6 ± 1.67	100.0 ± 0.00	-	-	-	-	-	-	-	-
90	6	74.	80.	86.	90.	96.	9	10	-	-	-	-	-	-	-	-	-	-

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	5.0 ± 2.91	4 ± 2.30	0 ± 2.91	4 ± 2.07	2 ± 2.38	6 ± 2.07	8.4 ± 2.07	0.0 ± 0.00										
100	72.8 ± 1.92	76.4 ± 2.07	84.0 ± 1.58	96.0 ± 2.91	99.0 ± 1.22	100.0 ± 0.00	-	-	-	-	-	-	-	-	-	-	-	-

Table 1.4. Effect of *P. pinnata* extract on the mortality of *M. hirsutus*at nymphal stage.

Concentrations (%)	**Mortality (%)**																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Control	-	-	-	15.0 ± 3.16	15.4 ± 3.50	23.6 ± 4.15	32.6 ± 5.3	45.4 ± 2.96	45.4 ± 2.96	45.4 ± 2.96	45.4 ± 2.96	45.4 ± 2.96	45.4 ± 2.96	-	-	-	-	-
10	18.8 ± 2.38	34.4 ± 2.70	40.8 ± 2.77	50.0 ± 2.54	57.0 ± 2.54	70.6 ± 2.88	76.2 ± 2.77	82.8 ± 1.92	87.6 ± 1.67	91.06 ± 3.04	97.0 ± 1.58	97.6 ± 2.07	100.0 ± 0.0	-	-	-	-	-
30	29	42	51	56.	62	71.	78.	88.	96	10	-	-	-	-	-	-	-	-

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	.6 ± 3.78	.6 ± 3.84	.0 ± 2.91	6 ± 2.60	.0 ± 3.53	6 ± 3.20	6 ± 3.64	2 ± 3.27	.4 ± 2.07	0.0 ± 0.0								
50	44.0 ± 3.67	52.8 ± 3.34	61.0 ± 3.53	67.6 ± 2.07	74.6 ± 3.36	82.0 ± 2.91	92.06 ± 3.04	100.0 ± 0.0	-	-	-	-	-	-	-	-	-	-
70	58.0 ± 3.53	65.6 ± 2.70	73.0 ± 2.44	79.0 ± 2.94	84.8 ± 2.16	90.8 ± 2.16	100.0 ± 0.0	-	-	-	-	-	-	-	-	-	-	-
90	71.4 ± 2.70	78.8 ± 2.38	83.4 ± 2.70	90.0 ± 2.91	97.6 ± 2.07	100.0 ± 0.0	-	-	-	-	-	-	-	-	-	-	-	-
100	85.8 ± 2.38	94.4 ± 2.07	98.6 ± 1.14	100.0 ± 0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 1.5. Effect of *O. sanctum* extract on the mortality of *M. hirsutus* adult bugs.

Concentrations (%)	**Mortality (%) / days**													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Contr	-	-	-	6.6	29.	44.	53.	63.2	84.0	91.0	96.0	100.	-	-

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ol				± 2.40	8 ± 6.76	4 ± 3.36	8 ± 3.19	± 2.58	± 2.91	± 3.39	± 2.23	0 ± 0.0		
10	5.6 ± 2.88	11.2 ± 3.34	21.2 ± 3.89	33.6 ± 3.64	41.4 ± 3.36	43.2 ± 3.03	48.4 ± 4.50	61.8 ± 4.43	72.4 ± 2.88	80.6 ± 3.04	86.2 ± 3.83	95.2 ± 3. 56	98.0 ± 1.58	100.0 ± 0.0
30	8.4 ± 2.30	11.2 ± 2.86	22.2 ± 3.49	35.2 ± 3.96	45.0 ± 2.91	53.4 ± 2.70	61.6 ± 2.70	78.8 ± 3.89	82.8 ± 1.92	88.4 ± 3.04	96.4 ± 2.07	97.80 ± 1.30	100.0 ± 0.0	-
50	13.4 ± 3.04	21.4 ± 2.50	30.0 ± 3.67	39.4 ± 4.15	52.4 ± 2.88	63.2 ± 3.83	74.2 ± 3.56	84.0 ± 3.16	90.2 ± 2.38	95.8 ± 2.58	100.0 ± 0.0	-	-	-
70	16.2 ± 2.58	23.6 ± 2.70	33.0 ± 3.53	44.4 ± 3.97	55.4 ± 2.70	68.8 ± 2.58	80.4 ± 3.20	90.6 ± 3.36	95.6 ± 2.40	98.0 ± 1.58	100.0 ± 0.0	-	-	-
90	19.8 ± 2.68	32.0 ± 4.12	40.4 ± 3.04	47.6 ± 2.70	60.4 ± 2.70	75.4 ± 4.03	84.4 ± 3.04	93.2 ± 3.56	97.2 ± 2.16	100.0 ± 0.0	-	-	-	-
100	23.6 ± 2.5	35.0 ± 2.6	42.2 ± 2.5	51.0 ± 2.9	64.0 ± 3.0	81.6 ± 2.8	92.2 ± 3.4	95.67 ± 4.34	100.0 ± 0.0	-	-	-	-	-

	0	4	8	1	8	8	2						

Table 1.6. Effect of *P. pinnata* extract on the mortality of *M. hirsutus* adult bugs.

Concentrations (%)	Mortality (%) / days												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	-	-	-	6.6 ± 2.40	29.8 ± 6.76	44.4 ± 3.36	53.8 ± 3.19	63.2 ± 2.58	84.0 ± 2.91	91.0 ± 3.39	96.0 ± 2.23	100.0 ± 0.0	-	-
10	7.2 ± 2.86	13.4 ± 1.94	22.8 ± 3.34	31.4 ± 2.70	38.8 ± 3.03	46.0 ± 2.91	54.0 ± 2.91	63.8 ± 2.58	73.4 ± 2.96	80.0 ± 3.53	94.2 ± 5.35	97.2 ± 1.78	99.2 ± 1.30	100.0 ± 0.0
30	8.00 ± 2.23	16.6 ± 1.67	26.6 ± 2.40	34.2 ± 3.03	41.2 ± 3.70	51.0 ± 2.73	60.4 ± 2.70	68.0 ± 2.23	78.4 ± 3.04	84.0 ± 2.73	96.6 ± 2.07	100.0 ± 0.0	-	-
50	11.4 ± 2.30	18.0 ± 2.34	27.2 ± 2.38	40.6 ± 2.40	48.0 ± 2.23	54.8 ± 2.58	63.8 ± 2.86	70.2 ± 2.58	81.2 ± 5.93	95.4 ± 2.07	100.0 ± 0.0	-	-	-
70	16.0 ± 2.54	24.0 ± 2.73	32.4 ± 3.64	43.4 ± 3.04	53.8 ± 2.86	61.8 ± 2.38	70.2 ± 3.49	83.2 ± 3.83	96.8 ± 1.92	100.0 ± 0.0	-	-	-	-
90	23.8 ± 2.86	32.0 ± 3.67	43.0 ± 2.91	54.0 ± 2.73	62.4 ± 2.88	72.8 ± 3.56	83.0 ± 2.91	96.2 ± 2.58	100.0 ± 0.0	-	-	-	-	-
100	27.0 ± 1.58	36.0 ± 1.58	45.2 ± 2.86	58.8 ± 3.96	78.0 ± 4.52	97.0 ± 1.58	100.0 ± 0.0	-	-	-	-	-	-	-

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Table 1.7. Effect of plants extract on the fecundity of *M. hirsutus.*

Concentrations (%)	*O. sanctum*	*P. pinnata*
Control	486 (100%)	486 (100%)
10	425 ± 0.65 (87.44)	405 ± 0.58 (83.33)
30	356 ± 0.78 (73.25)	317 ± 0.83 (65.22)
50	284 ± 0.45 (58.43)	258 ± 0.64 (53.08)
70	196 ± 0.97 (40.32)	164 ± 0.47 (33.74)
90	87 ± 0.89 (17.90)	64 ± 0.57 (13.16)
100	26 ± 0.81 (5.34)	13 ± 0.40 (2.67)

DISCUSSION

M. hirsutus (Green), also known as the pink mealy bug, is a significant pest that damages delicate leaves, produces tuliptre illnesses, and results in a quantitative loss of leaf production [22]. Because mulberry gardens are extremely sensitive to silkworms, use of pesticides with high toxicity and extended residual effects are banned [23].

Botanical pesticides are made up of a range of isolated secondary metabolites that exert physiological and behavioural impacts on agriculturally significant pests and illnesses, including growth suppression, oviposition, feeding deterrent, acute toxicity, and developmental disruption [24]. The purpose of this study was to determine how well aqueous leaf extracts from two medicinal plants O. sanctum and P. pinnata affected the hatchability of eggs, adult pink mealy bug (M. hirsutus) mortality, and fecundity in vitro.

The findings show that, in comparison to the control, the hatching of M.hirsutus eggs was delayed by 4 to 8 days in extracts of O. sanctum and prolonged by up to 12 days with P. pinnata. Hatching was completely inhibited at higher concentrations. The results of this investigation were consistent with those of Kathak and Pandey [25], who noted that one of the benefits of combining neem and eucalyptus was that the oil reduced the hatchability of C. cephalonica eggs. According to this finding, aeropyles tiny chorion holes associated with embryonic respiration allow secondary metabolites and their volatiles to penetrate eggs, preventing them from hatching [26,27, 28]. Govindaiah et al. [29] assessed the effectiveness of aqueous leaf extracts of three medicinal plants Eucalyptus globulus, O. sanctum, and P. betle against the hatching of eggs (M. hirsutus). According to their findings, E. globuls (20%) had the lowest rate of egg hatching at 100% concentration, followed by O. sanctum (30.42%) and P. betle (36.06%) compared to the control (96.73%). Using botanical extracts to combat a variety of insect pests, Gaspari et al. [30] found that adults fed a sucrose diet including extracts lay a much lower number of eggs with poor hatching rates.

The data from the aforementioned experiment made it abundantly evident that on the fourth day, P. pinnata extract caused 100% nymphal death, which was then followed by O. sanctum (6th day). The aforementioned data makes it abundantly evident that none of the nymphs reached adulthood because the extracts, at all doses, had a negative impact on their growth and development. The study's use of botanical extracts may have included some chemical components that can impede nymphal development and feeding. The nymph was not significantly affected by decreased concentrations, and it was comparable to the control. This can be the result of the extract's toxicants being diluted. Procera and O. sanctum botanical extracts were tested by Yousef et al. [31] against the cotton pink boll worm Pectinophoragossypiella (Saunds.). They clarified that the high concentration of lead, alkaloids, and flavonoids in the extract, which reach olfactory centres and are subsequently conveyed to the brain through sensory axons and influence the acetylcholine receptors in the nervous system, may be the cause of O. sanctum's very poisonous effect. Furthermore, pongapin and karanjin, two important flavonoids, are present in the Pongam leaf extracts [32, 33].

In the extracts evaluated at lower doses, the adult mortality rate was comparable to the control. At greater doses (100%) 100% death was noted with P. pinnata extract on day 7, whereas O. sanctum showed 100% mortality on day 9. In order to control mealy bugs and leaf rollers, Maheswari and Govindaiah [34] investigated the pesticidal activity of plant extracts, specifically Lantana camara L. (lantana), Allium sativum L. (garlic), Zingiber officinala Rose (ginger), Azadirachta indica Juss. (neem), and Vitex negundo L. (vitex). The outcome showed that, when compared to other extracts that are safer and cause no harm to silkworms, the lantana extract at lower concentrations (2% and 11%)was shown to be better in controlling mealy bugs and leaf rollers. Groups of cysteine protease enzymes, including

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papain, alkaloids, terpenoids, flavonoids, and non-protein amino acids, are present in the Carica papaya leaf extracts and are harmful to pest plant sucking insects, such as aphids, spotted bollworms, mealy bugs, and whiteflies [35]. According to Govindaiah et al. [36], at greater concentrations, adult mortality from E. globules was 100% on day eight; whereas, for O. sanctum and P. betleit, it was on days nine and eleven, respectively. The rate at which plant extracts were applied determined their adulticidal properties. In comparison to the nymphal stage, the extracts were less efficient during the adult stage. This could result in the development of a protective covering known as mealy substance on the adult bug's body. This substance is produced by the epidermal wax gland and is delivered to the body's surface by ducts, pores, and other kinds of secretory seats [37]. According to Tanwar et al. [38], adult mealy bugs have cryptic habitats in plants and a waxy layer covering their bodies that makes them resistant to water, making contact botanical pesticides less effective against them.

In relation to the extracts' ovipositon deterrent properties, adult mealy bug fertility decreased as concentration increased. P. pinnata extract (13 eggs) showed the greatest drop in fecundity, whereas O. sanctum (26 eggs) showed the second-most reduction. According to Pandy et al. [39], the bioactive components of Ocimum sp., such as euginol, mono and sesquiterpenoids, are generally oviposition deterrent.Additionally, according to Yadav and Bhargava [40], P. pinnata has been shown to inhibit the oviposition of a number of insect pests. As a result, the early stages of infection require the application of botanical extracts.

CONCLUSION

When compared to O. sanctum, P. pinnata was the most effective extract out of the two evaluated to control mealy bugs. As a result, mulberry growers may choose to treat tukra infections in their mulberry gardens with botanical extracts rather than pesticides.

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