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Management of Two Major Sucking Pests Using Neem Oil Formulation

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Abstract

Present investigation was carried out to formulate an effective bio pesticide formulation against two major sucking pests of field crops. Neem oil, surfactant and cassava leaf extract were the three constituents used in the formulations. Major field pests, the papaya mealy bug, *Paracoccus marginatus* and cowpea aphid, *Aphis craccivora* were selected for the study. A total of six formulations at concentration of 2, 1 and 0.5 were sprayed on mealy bug infested papaya seedlings and aphid infested cowpea plants. The mortality percentage varied according to formulations and 1% formulation F which contained 50 ml neem oil, 30 ml surfactant and 20 ml cassava leaf extract proved to be most effective bio pesticide formulation against these sucking pests. Accordingly, the amount of neem oil required for control of these pests was reduced due to the additive action of other constituents.

Keywords: Sucking pests; Bio-pesticide formulations; Neem oil; Papaya mealy bug; Cowpea aphid; Cassava leaf extract

Introduction

Indian agriculture is the backbone of our economy and India's crop yield potential suffers loss at a rate of 30 million tons/year [1]. Perhaps, the vital reason for such shortage was the crop or yield loss incurred by various insect pests. Sucking pests including mealy bugs, aphids and whiteflies have ever been a menace to field crops and often severe pest out breaks minimize the crop production. They suck sap from the host and the nutrient deficient host plant become stunted, distorted, yellowish, and show reduced vigor and premature loss of leaves [2,3]. Copious secretion of honeydew by these pests provides a medium for the growth of black sooty mold, which is detrimental to the plant, as they cover on the leaves and impair photosynthesis and induce the plant in stress. Infestation can also inflict indirect damage to the host by transmitting pathogens, particularly an array of viruses [4,5].

Proper management of these pests serves to avoid crop losses and provide more economic back up for the farmers. India has vast pesticide market and about 3% of the total pesticides used in the world are utilized in India. Realizing the adverse effect of synthetic pesticides, there is a global awareness to employ alternate strategies, particularly with bio pesticides for the management of pest complexes in agro ecosystem.

Neem products has a pivotal role in the management of sucking pests especially mealy bugs and aphids and this has also been supported by earlier workers [6-8]. Due to its efficacy and anti-repellant activity, neem oil and its various formulations were widely used as a bio pesticide in India [9]. Even, neem based application of chemical insecticides against field pests were also recommended [10]. Application of neem oil with locally available detergent or surfactant against mealy bugs and aphids provided satisfactory control.

Cassava leaf and tuber rind which are estimated to be 5-7 tons on each harvest, are not fully utilized as these are the reservoir of high concentration of cyanoglucosides. Cassava leaf and tuber rind are very effective in the management of nematodes in okra [11] and various insect pests [12].

Indiscriminate application of oils on field crops creates several physiological setbacks to the treated plants. Moreover, availability of such bio pesticides is a major constrain and therefore their application dose has to be minimized. Adequate formulation of bio pesticides (i.e) bio pesticidal oils with certain fillers serves in reducing the amount of oil without affecting their effect.

Present study aims to prepare an effective formulation using locally available neem oil, surfactant and leaf extract from cassava against the papaya mealy bug, *Paracoccus marginatus* Williams and Granara de Willink and cowpea aphid, *Aphis craccivora* Koch. The results elaborated were an outcome of a series of trial experiments conducted using the three constituents used for the formulation.

Materials and Methods

Maintenance of target pests

Major sucking pests the papaya mealy bug, *P. marginatus* and cowpea aphid, *A. craccivora* were selected for the current investigation. Culture of mealy bugs was maintained on papaya seedlings at 30 ± 20°C, 70 ± 5% RH. Similarly, colonies of *A. craccivora* were maintained on the cowpea plants. Highly infested leaves were selected for treatment.

Preparation of cassava leaf extract

Extract was collected by steam distillation method. Fresh leaves (250 g) of cassava were taken in a pressure cooker of capacity 2 L containing 1 litre of water. It was closed and boiled, and the vapours released through its outlet were passed through a rubber tuber (dia. 0.5 cm) that was connected to a Leibigs condenser. After continuous cooling by circulating cold water, the extracts were collected.

Preparation of test solution

According to the trial experiments, three constituents – locally available neem oil, surfactant and cassava leaf extract were mixed at various combinations to form a 100 ml solution. Six formulations were tested in the present study and its combination was depicted in Table 1. Each formulation was diluted to 2, 1 and 0.5% for bioassay.

Formulation	Neem oil	Surfactant	Cassava leaf extract
A	80	10	10
B	60	20	20
C	20	40	40
D	30	30	40
E	40	30	30
F	50	30	20

Table 1: Amount of neem oil, surfactant and cassava leaf extract used for the formulation (ml)

Bioassay

The infested leaves were kept in Petri dishes and test solutions were sprayed using an atomizer. Population of the live insects was recorded before the spray and 1, 2, 3 days after treatment.

Percentage mortality = A-B/A × 100; where A = pre-treatment population of insects and B= post treatment population of insects.

Phytotoxicity test

Leaf burn was graded using the following scale rating scale as follows

1 - no burn

2 - 1-10% burn

3 - 11-25% burn

4 - 26-50 burn

5 - more than 50% burn

The leaf burn percentage due to spraying of formulation was based on the burn on the surface area to the total surface area.

Statistical analysis

Statistical analysis was done by Analysis Of Variance (ANOVA) in a random block design and pair-wise comparison was carried out by Duncan's Multiple Range Test (DMRT, $p \leq 0.05$) using SPSS 17.0.

Results

Mortality of papaya mealy bugs due to treatment of various formulations and the results of phytotoxic test done on papaya seedlings were shown in Tables 2 and 3 respectively. Formulation A and B produced cent percentage mortality at all the concentrations tested even one day after treatment. But, both the formulations caused severe leaf burn and the treated leaves dried even one day after treatment. Lower concentration (0.5%) of these formulations even produced leaf burn as shown in Table 3. It was observed in the subsequent observations that due to severe leaf burn, the vigour of the plant was lost. Even though both formulations killed all the mealy bugs, they completely failed to penetrate through the waxy coating of the papaya mealy bug which serves as a protection to this pest. Concerned with these formulations, since amount of oil was more with less surfactant the formulation did not mix well.

Formulation	Concentration of test solution (%)								
	2			1			0.5		
	Day After treatment, DAT								
	1	2	3	1	2	3	1	2	3
A	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}
B	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}	100.0^{g}
C	74.46^{d}	78.33^{d}	78.33^{d}	52.34^{c}	52.34^{c}	52.34^{c}	39.52^{b}	39.52^{b}	39.52^{b}
D	81.66^{e}	81.66^{e}	81.66^{e}	55.37^{c}	55.37^{c}	55.37^{c}	41.33^{b}	41.33^{b}	41.33^{b}
E	85.00^{e}	85.00^{e}	88.74^{e}	58.82^{c}	75.42^{d}	75.42^{d}	44.48^{b}	44.48^{b}	53.20^{c}
F	100.00^{g}	100.00^{g}	100.00^{g}	91.44^{f}	91.44^{f}	100.00^{g}	82.32^{e}	82.32^{e}	82.32^{e}
Control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

Table 2: Mortality (%) of *Paracoccus marginatus* due to the treatment of formulations. Means with the same letters are not statistically significant by Duncan's multiple range test ($p \leq 0.05$)

Formulation C and D produced negligible mortality at lower concentrations tested (1 and 0.5%) and there was no increase in mortality according to days after treatment. Formulation C killed 74.46% mealy bugs one day after treatment but mortality apparently increased to 78.33% at 3 DAT. Whereas, 2% of formulation D produced 81.66% mortality at all three observations. Formulation C at

2% produced almost 25% leaf burn three days after treatment whereas formulation D did not produced phytotoxic problem to the papaya seedlings, but both these formulations were not effective for the control of papaya mealy bug (Table 4).

Formulation	Concentration of test solution (%)								
	2			1			0.5		
	Day After treatment, DAT								
	1	2	3	1	2	3	1	2	3
A	5	5	5	5	5	5	5	5	5
B	5	5	5	5	5	5	5	5	5
C	1	2	3	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1	1
Control	1	1	1	1	1	1	1	1	1

Table 3: Phytotoxic grade of papaya seed lings due to the treatment of formulations. Means with the same letter are not statistically significant by Duncan's multiple range test ($p \leq 0.05$)

Formulation	Concentration of test solution (%)								
	2			1			0.5		
	Day After treatment, DAT								
	1	2	3	1	2	3	1	2	3
A	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}
B	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}
C	90.45^{d}	90.45^{d}	90.45^{d}	82.33^{c}	82.33^{c}	82.33^{c}	64.32^{b}	64.32^{b}	64.32^{b}
D	95.41^{d}	100.0^{e}	100.0^{e}	78.34^{c}	78.34^{c}	78.34^{c}	63.45^{b}	63.45^{b}	63.45^{b}
E	93.43^{d}	93.43^{d}	100.0^{e}	80.11^{c}	80.11^{c}	80.11^{c}	68.30^{b}	68.30^{b}	68.30^{b}
F	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	100.0^{e}	86.32^{c}	86.32^{c}	86.32^{c}
Control	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

Table 4: Mortality (%) of *Aphis craccivora* due to the treatment of formulations. Means with the same letter are not statistically significant by Duncan's multiple range test ($p \leq 0.05$)

Formulation E at concentration of 2% produced 85.0% effect which became 88.74 at 3 DAT. Lower concentrations, 1 and 0.5% produced 58 and 44% mortality at 1 DAT which became 75 and 53% mortality respectively at 3 DAT. Even though the formulation did not produce satisfactory control, it was able to wash the mealy substance without causing any leaf burn.

Spraying of 2% of formulation F killed all mealy bugs 1 DAT and treatment with 1% also produced same effect at 3 DAT. This formulation at 0.5% produced 82% mortality in all the three observations. This formulation washed out the mealy substance and exposed the insects which reflected in the increased mortality percentage compared with the formulation E. No leaf burn was observed in all observations even 2% was sprayed.

No mortality or leaf burn was recorded for control batches indicating that spraying of water won't kill papaya mealy bugs.

For aphids also formulation A and B produced cent percentage mortality at concentration of 0.5% also. But due to the severe burn observed in cowpea leaves due to the spraying, these formulations cannot be recommended. Formulations C, D and E produced same effect at all the concentrations tested. These formulation at 0.5% concentration produced mortality percentage ranging from 63-68%. Similarly, spraying 1% of these formulations killed 78-82% aphids. Formulation C at 2% concentration produced 90.45% mortality in all the three observations, but D at the same concentration killed 95% of insects and mortality increased to 100% at 2 DAT Aphid infested leaves of cowpea sprayed with 2% of formulation E neem oil caused cent percentage mortality on 3 DAT. Formulation C at 2%

concentration produced almost 25% leaf burn 3 DAT. Similarly formulation D and E at 2% also produced leaf burn as shown in Table 5. But these formulations at 1 and 0.5% when sprayed did not create leaf burn. Formulation F was effective against aphids also. Cent percentage mortality was obtained even at 1% of formulation F with no leaf burn. Control batch recorded no mortality or leaf burn as in case of papaya mealy bug.

Formulation	Concentration of test solution (%)								
	2			1			0.5		
	Day After treatment, DAT								
	1	2	3	1	2	3	1	2	3
A	5	5	5	5	5	5	5	5	5
B	5	5	5	5	5	5	5	5	5
C	1	2	3	1	1	1	1	1	1
D	1	1	2	1	1	1	1	1	1
E	1	2	2	1	1	1	1	1	1
F	1	2	2	1	1	1	1	1	1
Control	1	1	1	1	1	1	1	1	1

Table 5: Phytotoxic grade of cowpea leaves due to the treatment of formulations.

Discussion

Lack of data regarding the importance and control of key pests, including application of pesticides, has become a drawback in present crop protection practices [13]. As Karar et al. [14] stated, insecticides are the quick method for the control of insect pests, but dependency on pesticides has its own complications and such situation demands some alternate measures. Therefore, it is imperative to develop a holistic system to tackle pests in a more eco-friendly, economically viable and socially acceptable manner for the farmer. Plant products were previously employed in the mealy bug management programme [15]. The age-old practice of using plant products to protect agricultural products has a revisit now as they are recognized as economic, safe and sound, hazardless, non-toxic, non-residual, and highly effective against an array of insect pests.

The papaya mealy bug, *P. marginatus* has been spreading fast across globe in over 50 countries ever since its first record in the Caribbean islands during 2003. In India, the pest was first reported from Coimbatore during 2007 infesting papaya and since then the list of agricultural and horticultural crops damaged/infested by this invasive pest is growing at an alarming rate [16]. Similarly, the cowpea aphid, *A. craccivora* is a threat to cowpea growers in all over India and due to heavy infestation, young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shriveling of pods and finally resulting in yield reduction [17].

The individual botanicals are not able to control crop pests, when the pest pressure is high or when there is epidemic in the field. Therefore, a need was felt to have a reliable bio pesticide formulation (BPF), which could be applied even at the time of an epidemic, when insect or disease population is high under field conditions.

Neem products reduce the infestation of various insect pests in tea [18], okra [19] and cowpea [20]. Daane, Bentley [21] reported that three mealy bug species, *Pseudococcus maritimus Ehrhorn*, *Pseudococcus viburni* and *Pseudococcus longispinus* were successfully controlled using need based products. Most tuber crops including cassava and yam bean have insecticidal properties [22,23], but literature keeps silence their utility in the management of sucking pests. Present investigation was conducted to prepare an effective bio pesticide formulation using neem oil, surfactant and cassava leaf extract carried out by bioassay testing of 2 major sucking pests under laboratory conditions.

Formulation A and B (i.e) formulations having high amount of neem oil, killed all the insects even 1 day after treatment. But, it created severe leaf burn and even growth of the plant was affected. Moreover, amount of neem oil and surfactant used was not in a proportion as the formulation did not mix well, and it reflected in the high oil content over the treated hosts. As amount of neem oil was reduced, phytotoxic problem greatly reduced, whereas those formulations (C, D and E) did not produced satisfactory effect. Upon these findings, amount of neem oil used in formulation E was slightly increased and new formulation was tested. Consequently, formulation F at 1% produced significant results over the two pests with no leaf burn.

Other than the neonate, all nymphal stages and adult female of mealy bugs are protected by hydrophobic mealy substance which repels water or any spray fluids and act as a barrier to its predatory insects [24,25]. Present investigation leads to the conclusion that satisfactory control measure of the mealy bug is achievable only by exposing the larvae/adult female by removing its protective covering. Even though formulation A and B produced significant mortality, they failed to wash the mealy substance to expose the hidden individuals present, if any. Formulation F succeeded in clearing the waxy mealy substance and exposing the papaya mealy bugs.

Previous works points out that neem oil and various addictives were successfully employed in management of the sucking pests. Roy, Gurusubramanian [26] reported that application of neem formulation to tea plants provided more than 75% reduction of three sucking pests.

Whitney [27] reported that strong jet spray application of neem oil in soapy water reduced the population of cotton mealy bug, *Phenacoccus solenopsis*. Hussain et al. [6] recommended application of neem oil 4% for control of mealy bugs. Khaskheli [28] recommended application of neem oil with locally available detergent for managing cotton mealy bugs.

Various unpublished reports recommend spraying of water for the control of these two pests. But, present investigation clearly indicated that papaya mealy bug and aphids cannot be killed by application of water and it merely dislodged a very few number of these pests from their hosts.

Even though several bio pesticide formulations were recommended against these sucking pests, the present formulation will be novel as it is highly nature-friendly and economical.

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