International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(1): 2612-2616

Received: 09-11-2019
Accepted: 13-12-2019

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# *In vitro* evaluation of biorationals and their mixed compounds against the melon aphid *Aphis gossypii* Glover

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**DOI:** https://doi.org/10.22271/chemi.2020.v8.i1an.8666

**Abstract**
In recent days sucking pests are getting more attention due to their efficiency to withstand the chemical pesticides by various mechanisms like resistance and resurgence. Usage of chemical pesticides can be reduced by using the biorational pesticides. Biorationals are any type of insecticides active against pest populations, but relatively innocuous to non target organisms and therefore, helpful in biological control. Here some of the biorationals and their mixers are tested against aphids (*Aphis gossypii* Glover) under laboratory condition in order to find the best biorational pesticide and their combination which leads maximum mortality of aphids and could serve as a better alternative for the chemical pesticides. Four botanicals {Neem seed kernel extract (NSKE), Pongamia seed extract (PSE), Custard apple seed extract (CASE) and Asafoetida}, two entomopathogenic fungi (*Beauvaria bassiana* and *Lecaenicillium lecanii*), one organic oil product {Agricultural spray oil (ASO) @ 2ml/l} and five combinations of them has been tried. Among the combinations tested, Agricultural spray oil + Custard apple seed extract + Pongamia seed extract + Neem seed kernel extract (2:1:2:2) @5% has given higher cumulative mortality (100%) after 72 hrs of treatment. And the most efficient entomopathonenic fungus against *A. gossypii* was *Lecaenicillium lecanii* IOF 1 strain which gave the mortality percent of 83.40. The steps, results and the future aspects of the study are discussed briefly in this paper.

**Keywords:** Botanicals, Entomopathogenic fungi, *Aphis gossypii*, Mortality percentage

## Introduction
Sucking pests are creating alarming level of loss in the crop production both in the open field and greenhouse condition. Among the various sucking pests, heavy infestation by melon aphids or cotton aphids, *Aphis gossypii* Glover is reported to be common due to their higher reproducing capacity and their efficiency to get resistance against most of the chemical pesticides currently in use (Sedlacek and Townsend, 1990; Shipp *et al*., 1991; Bennison, 1992; Hassan *et al*., 2008) [32, 33, 4]. With the increase in the levels of aphids, it is reported that the fruit parameters of the vegetables were decreased causing enough damage to the economic yield (Yasarakinci and Hincal, 1997; Tehri *et al*., 2014) [41, 36]. Biorationals are any type of insecticides active against pest populations, but relatively innocuous to non target organisms and therefore, non-disruptive to biological control i.e. plant extracts, insect pathogens, etc (Crump *et al*., 1999, Eilenberg *et al*., 2001, Ware and Whitacre 2004) [7, 9, 40]. Under both lab and field conditions the neem products were reported to be most effective against sucking pests especially against aphids (Ascher *et al*., 1984; Lowery *et al*., 1993; Kulat *et al*., 1997; Biswas, 2013; Chaudhary *et al*., 2017) [2, 23, 18, 5, 6]. Several workers reported pongamia seed extract as an effective pesticide against large number of insect pests (Stein and Klingauf 1990; Katole *et al*., 1993; Hiremath *et al*., 1997, Kumar and Singh, 2002, Elena *et al*., 2014) [34, 16, 15, 19, 10]. Oil extracts from the seeds of sugar apple (Cashew) tested in both laboratory and field trials have shown the immense potential of cashew seed extracts to control the major sucking pests of greenhouse such as silver whiteflies, aphids and mites due to the active principle Neoannonin present in it (Kawazu *et al*., 1989; Rupprecht *et al*., 1990; Lin *et al*., 2009) [17, 29, 21]. There are also studies stating that combination of botanicals causing major breakthrough in the pest management like pongamia and neem seed extracts mixed together have also shown synergetic effect against two spotted spider mites and aphids in both laboratory and field

condition studies (Rao *et al*., 2002; Venkatesan *et al*., 2007) [28, 38]. Vignesh *et al*., 2019 also found promising results when they combined Pongamia, Neem, cashew seed extracts and the organic oil against two spotted spider mites. The mixed extracts of Pongamia +aloe + NSKE recorded higher mortality (77.6%) followed by agave + chilli (71.13%) and NSKE (65.44%) which was more than any of the botanicals used alone under the laboratory condition (Barapatre, 2001; Loganathan *et al*., 2006) [3, 22]. *Beauveria bassiana* and *Lecanicillium lecanii* are the two most important entomopathogenic fungi tested against most of the sucking pests and proved to be effective in causing the mortality (Ugine *et al*., 2005; Mhazo *et al*. 2011; Erler *et al*., 2013; Dogan *et al*., 2017) [37, 24, 11, 8]. By finding the best biorational combinations it will not only form the alternate tool for chemical pesticides but also be useful in the formation of integrated pest management strategies for the eco-friendly pest management.

## Material and Methods

In vitro evaluation of different biorationals against aphids was done in the laboratory condition of Department of Entomology, UAS, Dharwad during the two seasons of 2017 & 2018.

### 3.4.2 Experimental layout

Location: Laboratory, Department of entomology, UAS, Dharwad.
Treatments: 13
Replications: 3
Experimental design: CRD

**Table:** Treatment details

| Treatment Details | | Trade name | Dosage | Source |
|---|---|---|---|---|
| T1 | *Beauvaria bassiana* | | 108 CFU/ ml | IOF, UAS,DWD |
| T2 | *Lecaenicillium lecanii* | | 108 CFU/ ml | IOF, UAS,DWD |
| T3 | Hing (Asafoetida) | - | 0.125% | Prepared |
| T4 | Neem seed kernel extract (NSKE) | - | 5% | prepared |
| T5 | Pongamia seed extract (PSE) | - | 5% | prepared |
| T6 | PSPE+NSKE (1:1) | - | 5% | prepared |
| T7 | Custard apple seed extract (CASE) | - | 5% | prepared |
| T8 | CASE+NSKE (1:1) | - | 5% | prepared |
| $T_9$ | CASE+PSE (1:1) | - | 5% | prepared |
| $T_{10}$ | CASE+PSE+NSKE (1:2:2) | - | 5% | prepared |
| $T_{11}$ | Agro spray oil (ASO) | SPELL | 2 ml/l | Vinayak oil industries, Mumbai |
| $T_{12}$ | ASO+ CASE+PSP+NSKE (2:1:2:2) | - | 5% | prepared |
| $T_{13}$ | Standard check Spinosad 45 SC | Tracer | 0.5 ml/l | Dow Agro Sciences |
| $T_{14}$ | Untreated control | | | |

## Essential biopesticides

For the preparation of various botanicals and their combination the standard procedure followed by Vignesh *et al*., 2019 [39] is used.

## Source of inoculums

Aphid population was maintained in the parthenocarpic cucumber plants grown under greenhouse without spraying any chemical in Hi-Tech horticulture farm, UAS, DWD.

## Treating aphids with biorationals

Parthenocarpic cucumber leaves were used for the treatment. First the leaves collected from the pesticide free field were washed and cutted into the circular shape with the size of petriplates to fit inside them. Then those leaves were dipped inside the prepared chemicals and followed by which they were kept above the mesh cloth for a while to remove the excess water on the leaves surface. Then the treated leaves were shifted to the petriplates. Three replications of twenty five aphids and mites adults were taken separately and were provided with the leaves treated within the petriplates. The per cent mortality of aphids was calculated starting from one day after the treatment (DAT). Aphids which weren't shown any movement while touched using the camel brush is taken as dead or mortal.

## Observations

1. Observations were made at one DAT at an interval of 24 hr till 72 hrs.
2. Per cent mortality of aphids and mites were calculated and corrected using the Schneider-Orelli's correction factor formula.

$$\text{Mortality percentage} = \left\{ \frac{\text{Total number of aphids- Dead aphids}}{\text{Total number of aphids}} \right\} \times 100$$

$$\text{Corrected morality percentage} = \frac{\text{Mortality \% in treated - Mortality \% in control}}{\text{100 - Mortality \% in control}} \times 100$$

## Statistical analysis

The data on mean population of insects were subjected to arc sin transformation and analyzed statistically. The treatment mean values were compared by LSD at 5 per cent probability to access the effective treatment (Gomez and Gomez, 1984).

## Results

Results of laboratory studies on the efficiency of various biorationals tested on aphids (*A. gossypii*) during 2017 and 2018 is presented on the Table 1.

**Table 1:** *In vitro* evaluation of biopesticides and botanicals bio efficacy on *A. gossypii* (2017) & (2018)

| Treatment | Corrected mortality (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2017 | | | 2018 | | | Pooled | | |
| | 24hrs | 48hrs | 72hrs | 24hrs | 48hrs | 72hrs | 24hrs | 48hrs | 72hrs |
| $T_1$: *Beauveria bassiana* @ $10^8$ CFU/g | 22.54 (28.33)$^e$ | 39.52 (38.93)$^e$ | 64.86 (53.62)$^e$ | 23.20 (28.79)$^d$ | 40.62 (39.58)$^f$ | 65.76 (54.17)$^e$ | 22.87 (28.56)$^d$ | 40.07 (39.26)$^f$ | 65.31 (53.89)$^f$ |
| $T_2$: *Lecanicillium lecanii* @ $10^8$ CFU/g | 33.97 (35.63)$^c$ | 60.43 (50.99)$^d$ | 82.90 (65.55)$^c$ | 36.78 (37.32)$^b$ | 61.09 (51.39)$^e$ | 83.90 (66.321)$^c$ | 35.37 (36.48)$^c$ | 60.76 (51.19)$^e$ | 83.40 (65.93)$^c$ |
| $T_3$: Hing (*Asafoetida*) @ 0.125 % | 27.14 (31.38)$^d$ | 35.02 (36.26)$^e$ | 41.90 (40.32)$^e$ | 29.33 (32.78)$^c$ | 33.68 (35.46)$^h$ | 40.95 (39.78)$^f$ | 28.24 (32.09)$^d$ | 34.35 (35.86)$^g$ | 41.43 (40.05)$^g$ |
| $T_4$: Neem seed kernel extract (NSKE) @ 5% | 40.39 (39.44)$^b$ | 63.90 (53.05)$^d$ | 76.27 (60.82)$^d$ | 45.51 (42.41)$^a$ | 64.57 (53.45)$^d$ | 75.60 (60.37)$^d$ | 42.95 (47.05)$^b$ | 64.24 (53.25)$^d$ | 75.93 (60.60)$^d$ |
| $T_5$: Pongamia seed extract(PSE) @ 5% | 36.19 (36.97)$^c$ | 58.43 (49.83)$^d$ | 64.19 (53.22)$^e$ | 38.52 (38.35)$^b$ | 56.33 (48.62)$^e$ | 65.09 (53.76)$^e$ | 37.36 (37.66)$^c$ | 56.88 (48.93)$^e$ | 64.64 (53.49)$^f$ |
| $T_6$: PSE + NSKE (1:1) @ 5% | 61.43 (51.58)$^a$ | 72.93 (58.62)$^c$ | 79.45 (63.02)$^c$ | 52.41 (46.36)$^a$ | 73.38 (58.91)$^c$ | 80.41 (63.70)$^c$ | 56.92 (48.96)$^a$ | 73.16 (58.77)$^c$ | 79.93 (63.36)$^c$ |
| $T_7$: Custard apple seed extract (CASE)@ 5% | 36.35 (37.06)$^c$ | 67.71 (55.35)$^c$ | 70.11 (56.83)$^d$ | 37.57 (37.79)$^b$ | 65.33 (54.91)$^d$ | 71.37 (53.932)$^e$ | 36.96 (37.43)$^c$ | 68.22 (55.66)$^d$ | 70.74 (57.23)$^e$ |
| $T_8$: CASE + NSKE (1:1) @ 5% | 51.00 (45.55)$^b$ | 70.62 (57.15)$^c$ | 79.05 (62.73)$^c$ | 52.44 (46.38)$^a$ | 69.81 (56.65)$^c$ | 77.71 (61.806)$^d$ | 51.72 (45.97)$^b$ | 70.21 (56.90)$^c$ | 78.38 (62.27)$^d$ |
| $T_9$: CASE+ PSE (1:1) @ 5% | 55.22 (47.98)$^b$ | 65.50 (54.01)$^c$ | 75.52 (60.32)$^d$ | 54.78 (47.72)$^a$ | 64.17 (53.21)$^d$ | 76.52 (60.99)$^d$ | 55.00 (47.85)$^a$ | 64.84 (53.61)$^d$ | 76.02 (60.66)$^d$ |
| $T_{10}$:CASE + PSE+ NSKE (1:2:2) @ 5% | 64.69 (53.52)$^a$ | 80.44 (63.72)$^b$ | 83.01 (65.63)$^c$ | 57.33 (49.20)$^a$ | 78.78 (62.54)$^b$ | 83.57 (66.06)$^c$ | 61.01 (51.34)$^a$ | 79.61 (63.13)$^b$ | 83.29 (65.85)$^c$ |
| $T_{11}$:Agricultural spray oil (ASO)@2 ml/l | 28.38 (32.18)$^d$ | 61.90 (51.86)$^d$ | 71.12 (57.47)$^d$ | 29.38 (32.81)$^c$ | 60.33 (50.94)$^e$ | 72.41 (58.29)$^d$ | 28.88 (32.49)$^d$ | 61.12 (51.40)$^e$ | 71.76 (57.88)$^e$ |
| $T_{12}$: ASO+ CASE + PSE+ NSKE (2:1:2:2) @ 5% | 36.87 (37.37)$^c$ | 98.51 (82.95)$^a$ | 100 (89.96)$^a$ | 39.60 (38.98)$^b$ | 96.99 (79.98)$^a$ | 100.00 (89.69)$^a$ | 38.24 (38.18)$^c$ | 97.75 (81.34)$^a$ | 100 (89.69)$^a$ |
| $T_{13}$: Spinosad 2.5 % SC @ 0.5 ml/l | 53.44 (46.95)$^b$ | 71.59 (57.76)$^c$ | 91.95 (73.49)$^b$ | 52.33 (46.32)$^a$ | 73.44 (58.96)$^c$ | 93.67 (75.39)$^b$ | 52.89 (46.64)$^b$ | 72.52 (58.36)$^c$ | 92.81 (74.42)$^b$ |
| S. Em± | 0.73 | 1.23 | 1.06 | 0.98 | 0.84 | 1.06 | 1.00 | 0.77 | 0.67 |
| CD (1%) | 2.86 | 4.83 | 4.17 | 3.85 | 3.30 | 4.16 | 3.93 | 3.01 | 2.58 |
| C.V | 3.34 | 4.20 | 3.24 | 4.51 | 2.90 | 3.21 | 4.25 | 2.45 | 1.85 |

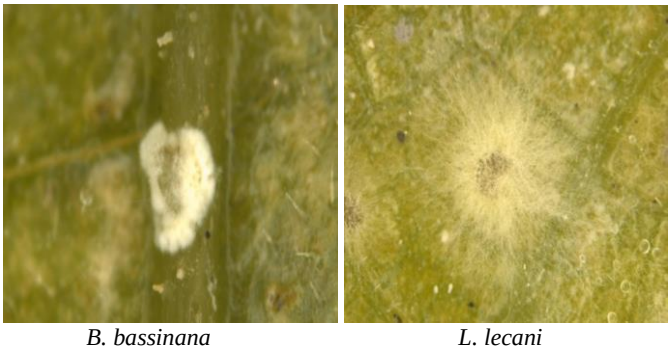
NSKE - Neem seed kernel extract, PSE- Pongamia seed extract, CASE- Custard apple seed extract, ASO- Agricultural spray oil
*Values inside the parenthesis are arc sin transformed.

**24 hrs after the treatment**

Among the different treatments tested, treatment ($T_{10}$) CASP + PSE+ NSKE (1:2:2) @ 5% gave higher mortality (64.69%) of aphids which was followed by ($T_9$) CASE+ PSE (1:1) @ 5% (55.22%), the chemical check Spinosad 2.5% SC @ 0.5 ml/l (T13) were statistically on par with each other in case of percent mortality caused. Combination products have shown more mortality than any individual biorational tested. When we look into the efficacy of two entomopathogenic fungi tested *L. lecanii* ($T_2$) produced maximum mortality (22.54%) of aphids. Among the individual botanicals tried, NSKE (T4) (40.39%) followed by CASE (T7) (36.35%), PSE (36.19%) have given maximum mortality and later two treatments were on par with each other. The treatment which has shown less effective after 24 hrs of treatment was *Beauveria bassiana* @ $10^8$ CFU/g ($T_1$) with the aphid mortality per cent of 22.54 during the in vitro study of 2017. Results obtained during 2018 *in vitro* study has few variations too, as all the combination treatments except $T_{12}$ was found to be on par with each other giving maximum aphid mortality which was on par with the chemical check $T_{13}$. Other results obtained during 2018 were similar with the studies conducted during 2017 which was also true with the pooled data of both the years.

**48 hrs after treatment**

Data obtained after 48 hrs after the treatment shows the change in the trend in the aphid mortality that $T_{12}$ (ASO+ CASE + PSE+ NSKE (2:1:2:2) @5%) produced maximum mortality of 98.51 per cent. Next best treatment after $T_{12}$ was T10 (CASP + PSE+ NSKE (1:2:2) @ 5%) with the recorded aphid mortality of 80.44 per cent which was followed by the chemical check ($T_{13}$). In case of individual botanicals tested CASE@5% ($T_7$) was leading in the mortality percentage (67.71%) followed by NSKE ($T_4$) @ 5% (63.90%) and PSE ($T_5$) @ 5% (58.43%) and the later two treatments were found to be statistically on par with each other. Bioefficacy of the entomopathogen *L. lecanii* ($T_2$) (60.43%) was nearly double that of *B. bassiana* ($T_1$) (39.52%) after 48 hrs of treatment. More or less similar result was obtained during the *in vitro* study of 2018. As it was also noticed that $T_{12}$ was leading treatment causing higher mortality (96.99%) followed by T10 (78.78%). And the least performing treatment during both the year study was T13 (Hing @0.125%) with the recorded aphid mortality per cent of 35.02 and 33.68 during 2017 and 2018 respectively.



*B. bassinana* *L. lecani*

**Fig 1:** Aphids infected with Entomopathogenic fungi in laboratory condition

**72 hrs after treatment**

After 72 hrs of treatment, $T_{12}$ produced cent per cent mortality of the aphids which was followed by the chemical check $T_{13}$ (91.95%). In case of entomopathogenic fungi tested $T_2$

(*Lecanicillium lecanii* @ $10^8$ CFU/g) had 82.90 per cent mortality of aphids and $T_1$ (*Beauveria bassiana* @ $10^8$ CFU/g) gave 64.86 per cent mortality. Among the individual botanicals tested $T_4$ gave more aphid mortality (76.27%) but it was statistically on par with $T_7$ with (70.11%) aphid mortality percentage. During 2018, *in vitro* study also the same trend had been followed as $T_{12}$ produced cent per cent mortality followed by the chemical check $T_{13}$. All other results were also found to be on par with the 2017 studies and the least effective treatment among the biorationals tested was $T_3$ with the aphid mortality percentage of 40.95. The pooled table of aphid mortality percentage during both the laboratory studies of 2017 and 2018 is presented on the Table 31 also clearly shows that $T_{12}$ was the best treatment among the treatments tested which was only treatment that had produced cent per cent mortality of test insect. More importantly it had over performed even the chemical check, Spinosad which had the aphid mortality percentage of 92.81.

The efficacy of various treatments as per the cumulative mortality after 72 hrs was in the order of: $T_{12}$ (ASO+ CASE + PSE+ NSKE (2:1:2:2) @ 5%)> $T_{13}$: Spinosad 2.5% SC @ 0.5 ml/l > $T_2$: *Lecanicillium lecanii* @ $10^8$ CFU/g >$T_{10}$ (CASE + PSE+ NSKE (1:2:2) @ 5%) > $T_8$ (CASP+NSKE (1:1) @ 5%)> $T_9$ (CASE+ PSE (1:1) @ 5%)> $T_4$: NSKE @ 5%> $T_{11}$ (ASO@2ml/l)> $T_7$ (CASE @ 5%)> $T_1$ (*B. bassiana* @ $10^8$ CFU/g)>$T_5$ (PSE @ 5%) >$T_3$(Hing @ 0.125%).

## Discussion

In case of *A. gossypii* the best biorationals combination which provided the maximum mortality of 100 per cent after 72 hrs of treatment is $T_{12}$ (ASO+ CASE + PSE+ NSKE (2:1:2:2) @5%) followed by the chemical check Spinosad 2.5% SC @ 0.5 ml/l (92.84%). This result is supported by Vignesh *et al*., 2019 [39] who also reported that the combination of neem, pongamia, custard apple seed extracts and mineral oil mixture produced the synergetic action against the mites which gave better result than the chemical check. Studies of Rao *et al*., 2002 [28] and Venkatesan *et al*., 2007 [38] was also in line with the present findings, as they also found the efficiency of using neem and pongamia extracts together.

In case of single botanicals tried NSKE caused more mortality of aphids (75.93%) followed by CASE (70.74%) after 72 hrs of treatment. It is similar with the findings of (Ascher *et al*., 1984; Lowery *et al*., 1993; Kulat *et al*., 1997; Biswas, 2013; Chaudhary *et al*., 2017) [2, 23, 18, 5, 6] who reported the same result as the present findings as neem to be a most effective pesticide against aphids and also there are similar demonstrations by (Schmutterer, 1990; Partridge and Borden, 1997; Tang *et al*., 2002) [31, 26, 35] stating that the active ingredient Azadiractin as reducing the survival period and fecundity of *A. gossypii* under laboratory condition. The efficiency of using custard apple seed extracts against sucking pests due to the active principle "Neoannonin" was also proved by several authors (Kawazu *et al*., 1989; Rupprecht *et al*., 1990; Lin *et al*., 2009) [17, 29, 21] which was also true with the present evaluation.

It is also clear that among the entomopathogenic fungi tried *Lecanicillium lecanii* IOF (Accession number: IOF KM215209) strain 1 is proved to be effective against *A. gossypi* which is also in line with the studies of various authors like (Saito, 1988; Ramakers, 1989; Gindin *et al*., 1996; Nirmala *et al*. 2006; Alavo, 2015) [30, 27, 12, 25] who reported *L. lecanii* as a best entomopathogen to be used against the sucking pests under greenhouse condition.

## Conclusion

Combination of biorationals produced better aphids mortality rather than going with the single botanicals. The entomopathogenic fungi *Lecanicillium lecanii* proved effective against aphids which have to be included in the integrated pest management programme along with the botanicals for improved results. Efficacy of using biorationals against *A. gossypii* is proved by the current study which has to be evaluated extensively under field condition in order to find the reliable alternative tool for prevailing chemical control measures.

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