Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 356-360

Received: 28-05-2019
Accepted: 30-06-2019

Vignesh M
Department of Agricultural Entomology, University of Agricultural, Sciences, Dharward, Karnataka, India

Patil RK
Department of Agricultural Entomology, University of Agricultural, Sciences, Dharward, Karnataka, India

Udhayakumar VS
Department of Agricultural Entomology, Tamil Nadu agricultural university, Tamil Nadu, India

Correspondence
Vignesh M
Department of Agricultural Entomology, University of Agricultural, Sciences, Dharward, Karnataka, India

Evaluation for promising biorationals and their synergetic combination against the two spotted spider mite *Tetranychus urticae* Koch

Vignesh M, Patil RK and Udhayakumar VS

Abstract
Two spotted spider mite *Tetranicus urticae* is major non insect pest of greenhouse crops causing huge setback for the cultivation. Problems with the chemical pesticides like resistance, resurgence and environmental effects diverted the pest management tactics towards eco-friendly manner. So the effort has been made under laboratory condition to find the best biorational pesticides and their possible combination which leads maximum mortality of the mite. Two entomopathogenic fungi (*Beauveria bassiana* and *Lecanicillium lecanii* IOF 1 strain @2g/l), four botanicals {Neem seed kernel extract (NSKE), Pongamia seed extract (PSE), Custard apple seed extract (CASE) @5% and Asafoetida @ 0.125%}, one mineral oil product {Agricultural spray oil (ASO) @5%} and five combinations of them has been tried. Among the combinations tried Agricultural spray oil + Custard apple seed extract + Pongamia seed extract + Neem seed kernel extract (2:1:2:2) @5% has shown synergetic action giving 100 percent mortality after 72 hours of treatment. And the most efficient entomopathonenic fungi against the two spotted spider mite was *Lecanicillium lecanii* IOF 1 strain which gave the mortality of 72.77 percent mites after 72 hours of treatment. Results and the future aspects of the current study are discussed briefly in this paper.

Keywords: *Tetranicus urticae*, biorationals, combinations, synergetic activity

1. Introduction

Two spotted spider mite (*Tetranicus urticae* Koch) is one of the major non insect pests threatening the cultivation of vegetable crops under greenhouse conditions all over the world [21, 13, 16] and in India [14]. Over reliance on chemical pesticides to control this pest leaded the problems like resistance, resurgence and the ecological imbalance [2, 9, 11, 38]. 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 [5, 7, 37]. In nature more than 1800 plant species are reported to have biopesticidal properties which was due to the bioactive phytochemicals such as alkaloids, terpenoids, poly acetylene, unsaturated isobutlyamide and retinoides etc. which may act as toxicant, repellent and behaviour modifiers [35]. Plant extracts like Neem, pongamia and custard apple seeds oil extracts were well studied and reviewed for their pesticidal properties [33, 12, 17, 19]. Synergism is defined as the interaction or cooperation of two or more substances to produce a combined effect greater than the sum of their separate effects [29]. Such synergetic activity was largely exploited in case of pyrethroids and was found highly effective but recently most of them were recorded with resistance throughout the world in varying levels [32, 23]. In case of biorationals there was no resistance recorded and the likelihood of resistance is also much lower as their extracts contain a group of active ingredients with diverse chemical nature [6, 31]. So if the successful synergistic compounds are found with the botanicals it will rectify the problem of both resistance creation and chemical contamination in the ecosystem. Pongamia and neem seed extracts mixed together have shown synergetic effect against two spotted spider mites [25, 36]. The mixed extracts of Pongamia +aloe + NSKE and recorded higher mortality (77.6%) of the larva followed by agave + chilli (71.13%) and NSKE (65.44%) which was more than any of the botanicals used alone under the laboratory condition [4, 20]. While looking into the next most important biorational product that is insect pathogens, Entomopathogenic fungi (EPF) multiply faster inside the greenhouse having the relative humidity factor. Among the EPFs *Verticillium lecanii* and *Beauveria bassiana* are found to be most effective entomopathogenic fungi especially against the sucking pests to reduce the pest load in natural friendly way giving higher mortality in self-perpetuating way [10, 34, 18]. So the current study is carried out under laboratory condition with the biorationals and their combination products on two spotted spider mite.

2. Materials and Methods

In vitro evaluation of different biorationals against two spotted spider mite (*Tetranicus urticae*) was done in the laboratory condition of Department of Entomology, UAS, Dharwad during the summer seasons of 2017 and 2018. Mite population was maintained on cucumber plants under the protected structures of Hi-Tech Horticulture farm, UAS, Dharwad.

2.1 Treatment details

T_1: *Beauveria bassiana* @ 10^8 CFU/g in 2g/l
T_2: *Lecanicillium lecanii* @ 10^8 CFU/g in 2g/l
T_3: Hing (Asafoetida) @ 0.125%
T_4: Neem seed Kernal extract (NSKE) @ 5%
T_5: Pongamia seed extract (PSE) @ 5%
T_6: PSE + NSKE (1:1) @ 5%
T_7: Custard apple seed extract (CASE) @ 5%
T_8: CASE + NSKE (1:1) @ 5%
T_9: CASE+ PSE) (1:1) @ 5%
T_{10}: CASE + PSE+ NSKE (1:2:2) @ 5%
T_{11}: Agricultural spray oil (ASO) @ 2ml/l
T_{12}: ASO+ CASE + PSE+ NSKE (2:1:2:2) @ 5%
T_{13}: Spiromesifen (Oberon) 240 SC @ 1 ml/l

IOF 1 strains of *B. bassiana* and *L. lecanii* is collected from Organic farming unit, UAS, Dharwad. Agricultural spray oil (ASO) is purchased from Vinayak oil industries, Thane, Maharashtra.

2.2 Preparation of Botanicals

Neem Seed Kernel Extract (NSKE) 5%: 5 kg of powdered neem seed was soaked in 10 litres of water overnight and filtered through double layered khada cloth which was taken as stock solution. 5% solution was prepared from this stock by adding water. In the same way PSE and CASE 5% was also prepared. PSE+NSKE (1:1): 2.5 ml of PSE and 2.5 ml of NSKE are mixed and 95 ml of water is added to make up 100 ml of 5% solution. CSPE+NSKE (1:1) @5% and CASE+PSE (1:1) @5% were also made in the same way. CASE+PSE+NSKE (1:2:2): From the stock solution of CASE, PSE and NSKE solution of 1ml, 2ml and 2ml were respectively taken and mixed to which 95 ml of water is added to produce 5% solution of CSPE+PSPE+NSKE (1:2:2). ASO+ CASE+PSE+NSKE (2:1:2:2): From the stock solution of each botanicals, 5ml each was dissolved in 100 ml to give 5% solution and then it was mixed in the ratio of 2:1:2:2 to get the 5% solution of ASO+ CASE+PSE+NSKE (2:1:2:2).

3. Laboratory analysis

Cucumber leaves collected from the pesticide free field were used for the study by cutting them into the circular shape to fit inside the petri plates. Leaf dipping method [1, 26] was used to treat each botanicals with the leaves. Then the treated leaves were shifted to the petriplates with the cotton below to absorb the excess moisture. Three replications of twenty five mite adults were taken and were provided with the leaves treated within the petri plates. The Per cent mortality of mites was calculated starting from 24 hrs after the treatment till 72 hrs. Mites which weren't shown any movement while touched using the camel brush is taken as dead or mortal. Per cent mortality of aphids and mites were calculated and corrected using the Schneider-Orelli's correction factor formula.

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

$$\text{Corre} \left\{\frac{\text{Total number of insects- Dead insects}}{\text{Total number of insects}}\right\} \times 100$$

4. Results

Observations on the mortality percentage of treated mites were recorded from 24 hours after the treatment till 72 hours as mentioned earlier. Mortality percentage of mites in the various treatments during 2017 and 2018 were presented in the table 1 and their cumulative mortality is represented in Grap.1 Based on the data presented following results are obtained:

Table1: Mortality percentage of mites in various treatments under laboratory condition during 2017 and 2018.

	Corrected mortality percentage (%) (2017)			**Corrected mortality percentage (%) (2018)**		
Chemicals	**24hrs**	**48hrs**	**72hrs**	**24hrs**	**48hrs**	**72hrs**
T_1: *Beauveria bassiana* @ 10^8 CFU/g	8.78(17.23)^{h}	16.01(23.58)^{f}	40.33(39.41)^{h}	9.11(17.56)^{h}	17.01(24.35)^{i}	41.11(39.86)^{h}
T_2: *Lecanicillium lecanii* @ 10^8 CFU/g	12.91(21.05)^{g}	35.08(36.31)^{e}	73.33(58.89)^{d}	13.39(21.46)^{g}	33.74(35.50)^{g}	72.20(58.15)^{d}
T_3: Hing (*Asafoetida*) @ 0.125%	4.45(12.17)^{i}	11.78(20.07)^{g}	15.17(22.91)^{i}	3.72(11.12)^{i}	12.28(20.51)^{j}	13.85(21.84)^{i}
T_4: NSKE @ 5%	16.22(23.74)^{f}	31.11(33.89)^{e}	55.33(48.04)^{g}	17.07(24.39)^{f}	33.04(35.07)^{g}	56.33(48.62)^{g}
T_5: PSPE @ 5%	25.00(29.99)^{d}	41.21(39.92)^{d}	68.56(55.87)^{e}	24.12(29.40)^{d}	43.56(41.28)^{e}	70.33(56.97)^{e}
T_6: PSPE + NSKE (1:1) @ 5%	28.00(31.94)^{c}	59.45(50.43)^{c}	77.17(61.44)^{d}	29.05(32.60)^{c}	57.12(49.07)^{d}	76.51(60.98)^{d}
T_7: CASPE @ 5%	12.11(20.36)^{g}	28.36(32.17)^{f}	49.33(44.60)^{g}	11.88(20.15)^{g}	27.43(31.57)^{h}	51.44(45.81)^{g}
T_8: CASP + NSKE (1:1) @ 5%	21.97(27.94)^{e}	34.28(35.82)^{e}	60.67(51.14)^{f}	22.42(28.25)^{e}	32.27(34.60)^{g}	59.67(50.55)^{f}
T_9: CASP+ PSPE (1:1) @ 5%	25.00(29.99)^{d}	37.67(37.84)^{d}	62.14(52.00)^{f}	25.83(30.53)^{d}	37.98(38.03)^{f}	63.22(52.65)^{f}
T_{10}:CASP + PSP+ NSKE (1:2:2) @ 5%	39.11(38.69)^{b}	59.18(50.27)^{c}	83.63(66.11)^{c}	40.11(39.28)^{b}	59.51(50.46)^{c}	84.10(66.47)^{c}
T_{11}: ASO@2ml/l	29.39(32.81)^{c}	65.47(53.99)^{b}	68.39(55.76)^{e}	29.50(32.88)^{c}	63.46(52.79)^{c}	66.07(54.35)^{e}
T_{12}: ASO+ CASP + PSP+ NSKE (2:1:2:2) @ 5%	39.11(38.69)^{b}	90.82(72.34)^{a}	100(89.63)^{a}	41.41(40.04)^{b}	92.49(74.06)^{a}	100.00(89.63)^{a}
T_{13}: Spiromesifen (Oberon) 240 SC @ 0.5 ml/l	46.21(42.81)^{a}	67.75(55.37)^{b}	95.26(77.40)^{b}	45.46(42.38)^{a}	70.54(57.10)^{b}	98.24(82.34)^{b}
Control	0.00	0.00	0.00	0.00	0.00	0.00
CD (1%)	1.65	2.69	3.86	1.65	2.37	3.86
S. Em	0.42	0.69	0.98	0.56	0.66	0.98
C.V	2.77	3.04	3.33	2.62	2.69	3.27

NSKE - Neem seed kernel extract, PSE- Pongamia seed extract, CASE- Custard apple seed extract, ASO- Agricultural spray oil
*Number of mites used in each treatments were 25.
*Numbers inside the parenthesis were arc sin transformed.

24 hrs after treatment

Among the combination of botanicals used for the study, treatment (T_{12}) ASO+ CASE + PSE+ NSKE (2:1:2:2) gave higher mortality (40.26%) of mites which was statically on par with the treatment (T_{10}) CASE + PSE+ NSKE (1:2:2) @ 5% with mite mortality of 39.61%. Combination products have shown more mortality than any individual biorational tested. Between the two entomopathogenic fungi tested *L. lecanii* (T_2) produced maximum mortality (13.15%) of mites (Fig.1). The chemical check Spiromesifen (T_{13}) has shown highest mortality of mites amidst the treatments under study (45.84%) 24 hrs after the treatment. Among the single botanicals tried, pongamia (24.56%) followed by NSKE (28.52%) have given maximum mortality. The treatment which have shown less effective was Asafoetida (T_3) (4.08%).

48 hrs after treatment

After 48 hours of treatment the trend in the mite mortality changed that T_{12} (ASO+ CASE + PSE+ NSKE (2:1:2:2) @5%) has given maximum mortality of 91.66% over taking the chemical check Spiromesifen@ 1 ml/l (T_{13}) which was showing (69.15%) most effective after 24 hrs of treatment followed by ASO (Agricultural spray oil@ 2ml/l) was statistically on par with each other. In case of individual botanicals tested same trend is followed as PSE@ 5% (T_5) was leading in the mortality percentage (42.38%) followed by NSKE (T_4) @ 5% (32.07%) and CASE (T_7) @ 5% (28.36%). Bio efficacy of the entomopathogenic *L. lecanii* (T_2) (35.08%) was nearly double times of *B. bassiana* (T_1) (16.01%) after 48 hrs of treatment.

72 hrs after treatment

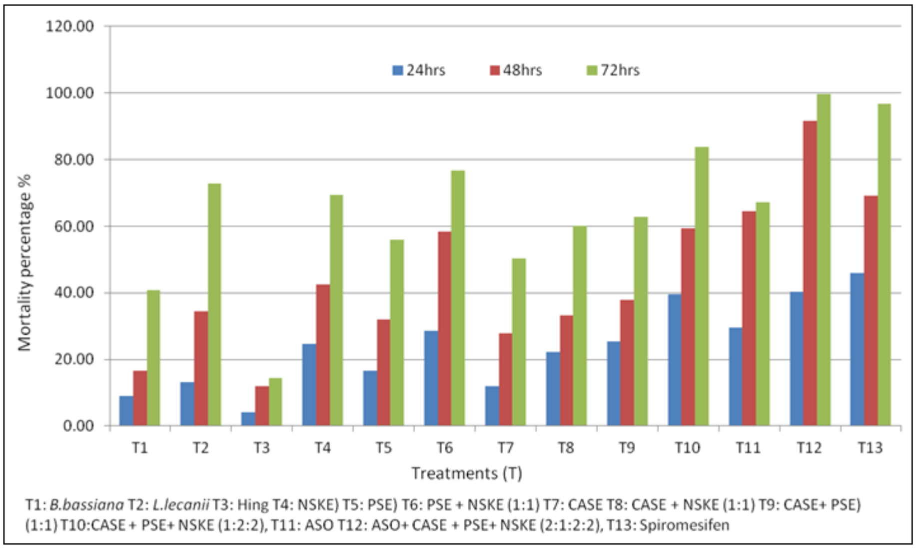
Cumulative mortality after 72 hours of the treatment shows that ASO+ CASE + PSE+ NSKE (2:1:2:2) @5% (T_{12}) gave 100% mortality which was followed by the chemical check Spiromesifen@1 ml/l (T_{13}) with 96.75% of mite mortality. PSE@ 5% (T_5) gave 55.83 percent mortality followed by NSKE gave 55.83% mortality when they were used singly. Between the entomopathogenic fungi tried *Lecanicillium lecanii* (T_2) gave cumulative mortality of 72.77% mortality after 72 hrs of treatment followed by *B. bassiana* (T_1)@2g/l which gave only 40.72% mite mortality.

L. lecanii on mite *B. bassiana* on mites

Fig 1: Entomopathogenic fungi infested two spotted spider mites

The efficacy of various treatments as per the cumulative mortality after 72 hrs was in order of:

T_{12} (ASO+ CASP + PSP+ NSKE (2:1:2:2) @ 5%)> T_{13} (Spiromesifen 240 SC @ 1 ml/l)>T_{10} (CASP + PSP+ NSKE (1:2:2) @ 5%)>T_6 (PSPE + NSKE (1:1) @ 5%)>T_2 (*L. lecanii* @ 10^8 CFU/g)>T_{11} (ASO@2ml/l)>T_5 (PSPE @ 5%)>T_9 (CASP+ PSPE (1:1) @ 5%)>T_8 (CASP + NSKE (1:1) @ 5%)>T_4 (NSKE @ 5%)>T_7 (CASPE @ 5%)>T_1 (*B. bassiana* @ 10^8 CFU/g)>T_3 (Hing @ 0.125%)



Graph 1: Cumulative mortality percentage of mites in various treatments under laboratory condition during test period.

5. Discussion

Based on the results obtained it is clear that combination treatments ended with more mortality percentage which is greater than their separate effects which indicates the presence of clear synergetic activity especially in case of neem, pongamia and custard apple seed extracts combination products which is in line with of Rao *et al*. [25] and Vinay *et al*. [36] who observed the synergetic activity when neem and pongamia extracts mixed together. Further when the oil product agricultural spray oil is mixed (ASO+ CASE+PSE+NSKE (2:1:2:2)) with them, the mortality rate was quickened and ending up with the 100 percent mortality 72 hours after the treatment which was producing the same result as with the studies of Elizabeth [8] who reported that when the mineral oil was mixed together with the botanicals the increased mortality of pests occur. Futher this study is also in line with Sabir *et al*. [27] who have mentioned the improved activity of Azadiractin when mixed with the agrospray oil. It was also clear that among the entomopathogenic tried *Lecanicillium lecanii* IOF strain 1 is proved to be effective against the two spotted spider mites causing more mortality than *B. bassina* as it is line with the studies of various authors [28, 24, 22, 3].

6. Conclusion

The current study gives the alternative tools for effective pest management by mixing the botanicals and mineral oil together. Including the effective entomopathogenic fungi with this, formation of integrated pest management programme to control the mite pests of any crops especially for the vegetable crops inside the greenhouse condition where the botanicals won't degrade faster and the entomopathogenic fungi activity is supported by the higher relative humidity. Further using these synergetic, eco-friendly and also effective compounds, pesticide load can be greatly decreased.

7. Reference

1. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18(1):265-266.
2. Al-Abbasi, Hassan S. Resistance to the two-spotted spider mite, *Tetranychus urticae* (Koch), in New Guinea Impatiens, Retrospective Theses and Dissertations, 1981, 7146.
3. Alavo TBC. The insect pathogenic fungus *Verticillium lecanii* (Zimm.) viegas and its use for pests control: a review. Journal of experimental biology and agricultural sciences. 2015; 3(4):16.
4. Barapatre AB. Evaluation of indigenous technology for management of *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Hub). M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad (India). July, 2001.
5. Crump NS, Cother EJ, Ash GJ. Clarifying the nomenclature in microbial weed control. Biocon Sci Tech. 1999; 9:89-97.
6. Coats JR. Risks from natural versus synthetic insecticides. Annu. Rev. Entomol, 39, 489-515.
7. Eilenberg J, Hajek A, Lomer C. Suggestions for unifying the terminology in biological control. Bio Control. 2001; 46:387-400.
8. ElizabethRBC.https://pdfs.semanticscholar.org/36ca/bdca891a7d26645f17e7107333922bc38bb0pdf. March, 2009.
9. Georghiou GP, Mellon RB, Saito T. Pest Resistance to Pesticides.Edn.1. Springer US, 1983, 987.
10. Gindin G, Barash I, Raccah B. The potential of some entomopathogenic fungi as biocontrol agents against the onion thrips, *Thrips tabaci* and the western flower thrips, *Frankliniella occidentalis*. Folia Entomological Hungarica. 1996; 57:37-42.
11. Helweg K. Fate of pesticides in surface waters, Laboratory and Field Experiments; Ministry of Environment, Danish Environmental Protection Agency, Pesticides Research, 2003, 68.
12. Hiremath IG, Joon AY, Soon K. Insecticidal activity of Indian plant extracts against *Nilaparvata lugens* (Homopera: Delphacidae). Applied Entomology and Zoology. 1997; 32:159-166.
13. Jeppson LR, Keifer HH, Baker EW. Mites injurious to economic plants. University of California Press, Berkeley, California, 1975, 614.
14. Kaur S, Srinivasan R, Cheema DS, Lal T, Ghai TR, Chadha ML, Monitoring of major pests on cucumber, sweet pepper and tomato under net house conditions in Punjab, India. Pest Manag. Horti. Ecosys. 2010; 16(2):148-155.
15. Kawazu K, Alcantara JP, Kobayashi A, Isolation and structure of neoannonin, a novel insectidal compound from seeds of *Annona squamosa*. Agric. Biol. Chem. 1989; 53:2719-2722.
16. Kono T, Papp CS. Handbook of agricultural pests. State of California Dept. of Food and Agric., Sacramento, California, 1977, 85.
17. Kulat SS, Nimbalkar SA, Hiwase BJ. Relative efficacy of some plant extracts against Aphis gossypii (Glover) and *Amrasca devastans* (Distant) on okra. PKV Research Journal. 1997; 21:146-148.
18. Labbe R, Gillespie DR, Cloutier C, Brodeur J. Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. Bio control Science and Technology. 2009; 19(4):429-446.
19. Lin C, Wu DC, Yu J, Chen B, Wang C, Ko W. Control of silverleaf whitefly, cotton aphid and kanzawa spider mite with oil and extracts from seeds of sugar apple. Neotropical Entomology. 2009; 38(4):531-536.
20. Loganathan J, Swaran D, Walia S. Efficacy of *Pongamia glabra* Vent. extracts on feeding and development of *Spodoptera litura* (Fabricius). Pesticide research journal. 2006; 18(1):15-19.
21. Mayer MKP. A revision of the Tetranychidae of Africa (Acari) with a key to genera of the world. Ent. Mem. Dep. Agri. Tech. Serv. Afri. 1974; 36:1-29.
22. Nirmala R, Ramanujam B, Rabindra RJ, Rao NS. Effect of entomofungal pathogens on mortality of three aphid species. Journal of Biological Control. 2006; 20(1):89-94.
23. Pereira AE, Souza D, Zukoff SN, Meinke LJ, Siegfried BD. Cross-resistance and synergism bioassays suggest multiple mechanisms of pyrethroid resistance in western corn rootworm populations. 2017; 12(6):e0179311. https://doi.org/10.1371/journal.pone.0179311
24. Ramakers PMJ, Biological Control in Greenhouses. In World Crop Pests (Editor in Chief: Helle W.); Aphids, Their Biology, Natural enemies and Control, eds. Minks AK, Harrewijn P. 1989; 2(C):199-208.
25. Rao NS, Rajendran R, Raguraman S. Antifeedant and growth inhibitory effects of neem in combination with sweet flag and pungam extracts on okra shoot and fruit borer *Earias vittella*. J. ent. Res. 2002; 26:233-238.
26. Robertson JL. Bioassays with Arthropods. 2nd ed., Boca Raton: CRC, 2007, 199.
27. Sabir N, Deka S, Singh B, Sumitha R, Hasan M, Kumar M *et al*., Integrated pest management for greenhouse cucumber: A validation under north Indian plains. Indian J. Hort. 2011; 68(3):357-363.
28. Saito T. Control of *Aphis gossypii* in greenhouses by a mycoinsecticidal preparation of *Verticillium lecanii* and effect of chemicals on the fungus. Japan Journal of Applied Entomology and Zoology. 1988; 32:224-227.
29. Saxena RC, Srivastava RC, Somani LL. Entomology at a glance Vol-1, Agrotech publishing academy, 2011, 146.
30. Sewify GH, Mabrouk AM. The susceptibility of different stages of citrus brown mite *Eutetranychus orientalis* Oudemans (family: Tetranychidae) to entomopathogenic fungus *Verticillium lecanii*. Proceedings and abstracts, Vth International Colloquium on Invertebrate Pathology and Microbial Control, Adelaide, Australia, 20-24 August, 1990, 395.

31. Silva-Aguayo G. Botanical insecticides, Redcliffe's IPM world textbook. University of Minnesota. https://ipmworld.umn.edu/silva-aguayo-botanical, 1994.
32. Smith LB, Kasai S, Scott JG. Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. Pesticide Biochemistry and Physiology. 2016; 133:1-12.
33. Stein U, Klingauf F. Insecticidal effect of plant extracts from tropical and subtropical species. Journal of Applied Entomology. 1990; 110:160-166.
34. Ugine TA, Wraight SP, Brownbridge M, Sanderson JP. Development of a novel bioassay for estimation of median lethal concentrations (LC_{50}) and doses (LD_{50}) of the entomopathogenic fungus *Beauveria bassiana*, against western flower thrips, *Frankliniella occidentalis*. Journal of invertebrate pathology, 2005; 89(3):210-218.
35. Verma RK, Chaurasia L. Alternate insecticides for termite control study and evaluation. Manthan (Hindi). 2003; 1:58-64.
36. Vinay K, Chandrashekar K, Sidhu OP. Synergistic action of neem and karanj to aphids and mites. J ENT Res. 2007; 31(2):121-124.
37. Ware GW, Whitacre DM. The Pesticide Book. MeisterPro Information Resources. Willoughby, OH, 2004, 488.
38. Zacharia JT. Ecological effects of pesticides, Pesticides in the modern world - Risks and benefits, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-458-0, In Tech, A vailable from: http://www.intechopen.com/books/pesticides-in-the-modern-world-risks-and-benefits/ecological-effects-ofpesticides, 2011.