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Potential of entomopathogens and neem oil against two emerging insect pests of vegetables

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ABSTRACT

Bioefficacy of different entomopathogenic microorganisms, viz *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, *Bacillus subtilis*-2 and botanical, i.e. neem oil were tested alone and their 1:1 combination with neem oil against two emerging insect pests of vegetables. Among the entomopathogens, *M. anisopliae* IIVR strain was most promising against hadda beetle (*Epilachna dodecastigmata*), followed by *M. anisopliae* commercial formulation, whereas *L. lecanii* was highly effective against painted bugs (*Bagrada hilaris*). However, neem oil was the most effective biopesticides against duo insect pests. The per cent mortality changed in time dependent manner and the lowest median lethal time (LT_{50}) for neem oil against *E. dodecastigmata* and *B. hilaris* were 45.09 and 102.03 h, respectively. Combinations of these entomopathogenic fungi (EPF) and neem oil (1:1) had lower LT_{50} values than each of their individual indicating the compatibility among them which was also confirmed through co-toxicity values greater than one in all the cases.

Key words: *Bagrada hilaris,* Compatibility, Entomopathogens, *Epilachna dodecastigma,* Median lethal time (LT_{50}), Native strains, Neem oil

In recent years, with changes in the cropping systems and climate, and introduction of highly input intensive high yielding varieties/hybrids, a paradigm shift in pest status has been observed in many crops including vegetable ecosystem. Some of the insect pests of vegetable crops which were previously considered as minor pests have become major and some are gradually attaining the major pest status in different regions of the country due to changes in the ecosystem and habitats (Rai *et al*. 2014). The emerging insect pests like hadda beetles, *Epilachna dodecastigma* (Wied.) and *Henosepilachna vigitioctopunctata* Fab. (Coccinellidae : Coleoptera) and painted bug, *Bagrada hilaris* (Burmeister) (Pentatomidae: Hemiptera) are gaining importance due to their expanding host-horizonand damage severity under the changing climatic conditions. Hadda beetles being polyphagous in nature, attacks vegetable crops like potato, brinjal, tomato and cucurbits (Kodandaram *et al*. 2014). Recently, its serious incidence was reported in cowpea and bitter gourd in many parts of Uttar Pradesh, Bihar and West Bengal. More than 80% leaves were infested by this beetle on cowpea during summer and *kharif* seasons in eastern part of Uttar Pradesh. Both grubs and adults feed on the leaves, flower and flower buds and thereby causing serious damage and the problem was much more severe when the crops were grown organically. Similarly, *B. hilaris* earlier causes minor damage to crops like rapeseed and mustard and at present; its serious damage was recorded on radish (*Raphanus sativus*) throughout its growth stage and more particularly during seed production stage imparting production of crimple and non-viable seeds. Both nymphs and adults occur in gregarious form and suck the sap from the tender leaves, buds, flowers and pods leading to sorting suitable management practices.

To control these pests, Indian farmers are commonly used to apply synthetic insecticides indiscriminately which may lead to problems like resistance to insecticides, resurgence of target insects and secondary pest outbreak in addition to these residues in food and beverages, contamination of groundwater, adverse effect on human health, and wide spread killing of non-target organisms (Halder *et al*. 2010, 2013). Hence, search for suitable and sustainable alternatives like bioagents especially entomopathogenic microorganisms are gaining importance due to their target specificity, self-perpetuity and obvious safety to the environment. The pest control prospects chiefly of entomopathogenic fungi (EPF), viz. *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium* (=*Verticillium*) *lecanii* and bacteria have been proved beyond doubt over the years. Another important consideration of these EPF as there is no report of development of any resistance. Among the botanicals,

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neem (*Azadirachta indica* A. Juss) is most widely used due to its diverse mode of action against wide range of insect pests across the agricultural and horticultural crops, easy accessibility and lower cost of production. Therefore, an attempt was made to control these duo emerging pests of vegetables by means of using biocontrol agents and botanical alone and also their 1:1 combinations for their compatibility and increased efficacy, if any.

MATERIALS AND METHODS

Talc based formulation of three entomopathogenic fungus, viz. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) both commercial formulation (1×10^8 cfu/g) and IIVR strain (1×10^{10}cfu/g), *Metarhizium anisopliae*(Metchnioff) Sorokin (Hypocreales: Clavicipitaceae) both commercial formulation (1×10^8 cfu/g) and IIVR strain (1×10^{10} cfu/g) and *Lecanicillium* (=*Verticillium*) *lecanii* R. Zare & W. Gams (Hypocreales: Clavicipitaceae) (2×10^9 cfu/g) commercial formulation and entomopathogenic bacterium *Bacillus subtilis*-2 (Ehrenberg 1835) (Bacillales: Bacillaceae) were taken for the experiments. Neem oil (1%) was prepared by dissolving in emulsifying water containing Triton-X-100 as an emulsifier. All the microbial insecticides (5 g/l of water) and neem oil alone and their 1:1 combinations with neem oil were tested for their efficacy against both the pests like hadda beetle and painted bug.

The painted bug infested leaves and twigs of radish were collected from the experimental farm of ICAR-Indian Institute of Vegetable Research (82°52' E longitude and 25°12' N latitude), Varanasi, Uttar Pradesh and stock culture was maintained in biocontrol laboratory (28 ± 2°C, 70–80 % relative humidity and a photoperiod of 13:11 (L:D) hour). 7±1 day old nymphs of *B. cruciferarum* obtained from the stock culture were used for bioassay experiments. Similarly, hadda beetle infested cowpea leaves collected from the seed production plots of the IIVR experimental farm and reared under laboratory condition as mentioned above and 7±1 day old grubs were used for the toxicological studies. Twenty insects of each test insects were placed in each petridish (9 cm diameter) and as such three replications were maintained in each case. Different microbial insecticides (1 ml), neem oil alone at their respective doses and their combination with neem oil (1:1) were sprayed under Potter's tower at 340 g/cm^2 pressure. The sprayed petridishes containing the treated test insects were fan-dried. Fresh uninfested and untreated plant materials were provided to their respective insects as food and kept at 27 ± 1°C and 70 ± 5 % RH.

For the assessment of toxic effect, mortality counts were taken at every 12 hr interval and moribund insects were considered as dead. The mortality data was corrected by Abbott's formula (Abbott 1925) and analysed by Probit analysis (Finney 1971) with SAS program (version 9.2). The control mortality, in almost all the cases, was below 10%. The median lethal times (LT_{50}) were determined and any two values were considered significantly different if their respective 90% confidence limits (CL) did not overlap.

To work out the compatibility of neem oil and these entomopathogens, co-toxicity coefficient (CC) was worked out by the following formula –

Co-toxicity coefficient = LT_{50} values of neem oil alone/ LT_{50} value of mixtures.

When the value of co-toxicity coefficient was >1 indicating they are compatible with each other and when it was < 1 showed that they are not compatible.

RESULTS AND DISCUSSION

Effect of different entomopathogens alone and their combination with neem oil

Marked differences were observed among these microbial insecticides alone and their combinations. The green muscardine fungus, *M. anisopliae* IIVR strain was found most promising entomopathogens against hadda beetle, *E. dodecastigmata* and the per cent protection over control (POC) were 60.33 and 85.53 at 4 and 6 days after treatment (DAT), respectively, followed by white halo fungus, *L. lecanii* and the corresponding values were 64.58, and 83.08 respectively (Table 1). However, *L. lecanii* was found most effective against the nymphs of painted bug, *B. hilaris* (48.80 and 69.05 POC at 4 and 6 DAT, respectively). In contrast, neem oil (1%) was the most

Table 1 Effect of different entomopathogens alone and their combination with neem oil (1:1) against *E. dodecastigmata* and *B. hilaris*

Biopesticides	Per cent protection over control			
	E. dodecastigmata		*B. hilaris*	
	4 DAT*	6 DAT	4 DAT	6 DAT
B. bassiana	43.55	58.74	38.57	58.49
M. anisopliae	47.92	66.08	38.88	59.11
B. bassiana IIVR strain	59.21	82.15	40.08	61.47
M. anisopliae IIVR strain	60.33	85.53	47.53	65.32
L. lecanii	64.58	83.08	48.80	69.05
B. subtilis - 2	46.11	64.97	35.46	56.75
Neem oil	69.56	87.08	48.95	69.64
B. bassiana + Neem oil (1:1)	73.89	92.67	75.41	88.70
M. anisopliae + Neem oil (1:1)	75.44	93.40	67.87	93.08
B. bassiana IIVR strain + Neem oil (1:1)	74.08	91.90	79.54	94.89
M. anisopliae IIVR strain + Neem oil (1:1)	77.98	94.33	79.16	95.83
L. lecanii + Neem oil (1:1)	72.59	91.25	78.21	93.17
B. subtilis – 2 + Neem oil (1:1)	56.46	81.77	44.83	65.01
CD (P=0.05)	3.55	2.97	3.69	4.18

*DAT= Days after treatment.

Table 2 Median lethal time of different entomopathogens and neem oil alone and their 1:1 combinations against *E. dodecastigmata*

Biopesticides	Heterogeneity		Regression equation (Y=)	LT_{50} (hr)	Fiducial limit	CC*
	df	χ2				
B. bassiana	6	1.811	2.611X – 0.362	113.19	124.75 – 102.70	
M. anisopliae	6	1.452	2.622X – 0.327	107.52	117.55 – 98.35	
B. bassiana IIVR strain	5	0.574	2.901X – 0.547	81.68	88.17 – 75.67	
M. anisopliae IIVR strain	6	1.047	2.733X – 0.123	60.86	69.38 – 53.39	
L. lecanii	4	0.228	2.311X + 0.797	65.95	75.68 – 57.47	
B. subtilis - 2	6	0.456	2.469X – 0.004	109.67	120.93 – 99.45	
Neem oil	6	3.527	2.32X + 1.163	45.09	49.50 – 41.06	
B. bassiana + Neem oil (1:1)	6	6.099	2.507X+0.841	44.96	49.74 – 41.80	1.003
M. anisopliae + Neem oil (1:1)	6	4.248	2.516X+0.888	43.07	47.07 – 39.41	1.047
B. bassiana IIVR strain + Neem oil (1:1)	6	7.800	2.267X + 1.268	44.31	48.78- 40.26	1.018
M. anisopliae IIVR strain + Neem oil (1:1)	5	1.004	1.786X + 2.269	33.85	41.04 – 27.92	1.332
L. lecanii + Neem oil (1:1)	5	0.726	2.162X – 1.453	43.71	48.52 – 39.37	1.032
B. subtilis – 2 + Neem oil (1:1)	3	1.181	2.795X – 0.311	79.39	98.12 – 64.23	0.568

*CC= Co-toxicity coefficient.

effective biopesticides across the duo emerging pests and the per cent protection over control at 4 and 6 DAT were 69.56 and 87.08 (*E. dodecastigmata*) and 48.95 and 69.64 (*B. hilaris*). When these entomopathogens were applied in combination with neem oil at 1:1 ratio the per cent protection over control were considerably higher in all the cases than their individual effect indicating compatibility among these major biopesticides. Highest control of 94.33% was recorded (Table 1) against 7 ± 1 day old grubs of *E. dodecastigmata* when half of recommended doses of each biopesticides was mixed, viz. *M. anisopliae* IIVR strain and neem oil (1:1 ratio) followed by 93.40% in case of combination of *M. anisopliae* commercial formulationand neem oil. The same trend was observed in *B. hilaris* also. Blending of *M. anisopliae* IIVR strain and neem oil gave highest protection against nymphs (79.16 and 95.83% POC at 4 and 6 DAT) of *Bagrada* followed by *B. bassiana* IIVR strainand neem oil. According to Ghelani *et al*. (2006) application of *V. lecanii* (2 × 10^8 cfu/g), *B. bassiana* (2 × 10^8cfu/g) and Vanguard (Azadirachtin 1 500 ppm) at 4 ml/litre proved effective and recorded per cent reduction between 50 and 70 against *Aphis gossypii* on Bt cotton. In another study Naik (2008) observed that oil based formulation of *B. bassiana* and *M. anisopliae* recorded 96.67% mortality of okra leaf hopper each, whereas *V. lecanii* recorded 96.77 and 97% mortality of aphids, and thrips respectively at 10 days after treatment. Compatibility of neem oil with *B. bassiana* was further confirmed and documented that there was no significant inhibition in vegetative growth of *B. bassiana* due to aqueous neem seed extract at 5% (Rodrıgues-Lagunes *et al*. 1997).

E. dodecastigmata

Significant differences were observed in the median lethal time of different entomopathogens and neem oil alone and their 1:1 combinations. The entomopathogenic fungi *M. anisopliae* IIVR strain was found most promising followed by *L. lecanii* when tested against 7 ± 1 day old grubs of *E. dodecastigmata* and their corresponding LT_{50} values were 60.86 and 65.95 hr respectively (Table 2). However, when neem oil (1%) was tested alone the median lethal time was just 45.09 hr and lowest amongst all the biopesticides. All these microbials insecticides when blended with neem oil (1:1 ratio) at their half-doses were found compatible and the corresponding LT_{50} values were 33.85, 43.07 and 43.71 hr for *M. anisopliae* IIVR strain , *M. anisopliae* commercial formulation and *L. lecanii*, respectively. Pathogenicity of *M. anisopliae* strains against coleopteran insects were confirmed by several authors in the past. Gindin *et al*. (2006) reported that *M. anisopliae* strains found pathogenic to all development stages of red palm weevil, *Rhynchophorus ferrugineus* causing up to 80–100% mortality of larvae and adult weevils under laboratory conditions. Swaminathan *et al*. (2010) who observed that neem oil 5% caused 60% adult mortality under laboratory conditions. In another study Vishwakarma *et al.* (2011) reported that *M. anisopliae* @ 3 g/l gave 70.71% reduction in *Epilachna* beetle population infesting bottle gourd.

B. hilaris

Neem oil (1%), amongst the all tested biopesticides, was recorded to be most effective in controlling the painted bug, *B. hilaris* infesting radish and the median lethal time was 102.03 hr, whereas among the entomopathogens *L. lecanii* had the lowest LT_{50} values (103.72 hr) followed by *M. anisopliae* IIVR strain (111.16 hr) and *B. bassiana* IIVR strain (121.42 hr), whereas when these bioagents were mixed with neem oil at 1:1 ratio all the entomopathogenic fungi were compatible with neem oil (Table 3). Lowest LT_{50} of 50.37 hr was recorded in *M. anisopliae* IIVR strain

Table 3 Median lethal time of different entomopathogens and neem oil alone and their 1:1 combinations against *B.hilaris*

Biopesticides	Heterogeneity		Regression Equation (Y=)	LT_{50} (Hr)	Fiducial Limit	CC*
	df	χ^2				
B. bassiana	3	2.918	6.989X – 9.733	128.20	150.16 – 109.45	
M. anisopliae	4	2.013	7.175X – 10.145	129.03	162.03– 102.76	
B. bassiana IIVR Strain	4	0.196	1.936X + 0.964	121.42	202.29 – 72.88	
M. anisopliae IIVR Strain	4	1.304	4.662X – 4.538	111.16	137.29 – 90.01	
L. lecanii	4	1.693	4.255X – 4.615	103.72	124.09 – 93.51	
B. subtilis - 2	4	1.212	4.319X – 4.761	131.96	151.08 – 111.91	
Neem oil (1%)	4	0.984	5.123X – 5.291	102.03	121.90 – 85.40	
B. bassiana + Neem oil (1:1)	6	0.413	2.976X – 0.169	64.58	77.69 – 54.01	1.580
M. anisopliae + Neem oil (1:1)	5	0.826	3.372X – 0.532	73.76	74.30 – 55.26	1.383
B. bassiana IIVR Strain + Neem oil (1:1)	4	2.989	4.793X – 2.712	60.62	67.48 – 44.75	1.683
M. anisopliae IIVR Strain + Neem oil (1:1)	3	3.329	7.657X – 6.575	62.48	67.10 – 58.44	1.633
L. lecanii + Neem oil (1:1)	5	0.215	4.860X – 3.273	50.37	58.06 – 43.70	2.026
B. subtilis – 2 + Neem oil (1:1)	4	0.326	2.170X + 0.552	112.07	229.33 – 54.77	0.910

*CC= Co-toxicity coefficient

+ Neem oil followed by *B. bassiana* IIVR strain + Neem oil (60.62 hr). The pathogenicity of *B. bassiana* and *M. anisopliae* against *B. hilaris* was verified and concluded that they caused good mortality or infection in Bagrada bug adults (Dara 2013). Our study is in conformity with the earlier study where neem based formulation nimbecidine has been reported compatible with *B. bassiana* and *V. lecanii* (Subbulakshmi *et al*. 2012). In another *in vitro* study Depieri *et al*. (2005) recorded the compatibility of emulsifiable neem oil (0.5, 1 and 1.5%), aqueous extracts of neem seeds (1, 2 and 4%) and leaves (0.15 and 1.5%) with *B. bassiana* and concluded that all the formulations had no effect on the fungus vegetative growth and on conidia production and viability. Many botanical insecticides including azadirachtin is having diverse mode of action. The apparent enhancement in activity of neem oil and entomopathogenic fungi mixtures were attributed to the possible additive, synergistic and/or stabilizing effect of neem oil (Halder *et al*. 2012 and 2013).

Among the entomopathogens, native strain of *M. anisopliae* was found most promising against hadda beetle, whereas *L. lecanii* was highly effective against nymphs of painted bug. However, among these duo emerging pests, *E. dodecastigmata* were found most susceptible than the *B. hilaris* to individual biopesticides. Form the present study it was evident that all the entomopathogenic fungi (EPF) were compatible with neem oil. The co-toxicity coefficient values of neem oil with *B. bassiana*, *M. anisopliae*, *B. bassiana* IIVR strain, *M. anisopliae* IIVR strain, *L. lecanii* were 1.003, 1.047, 1.018, 1.332 and 1.032, respectively against *E. dodecastigmata* indicating combination of these EPF and neem oil at 1:1 ratio were compatible. Similar trend was observed in *B. hilaris* also. However, when *B. subtilis*-2 was blended with neem oil and evaluated against *E. dodecastigmata* and *B. hilaris*, the mixture had CC value of lower than 1 (i.e., 0.568 and 0.910, respectively) depicting incompatibility of entomopathogenic bacterium with neem oil. Suitability of native/local strain of bioagents over conventional was reported by several authors. In our study also all the local strains of *M. anisopliae* and *B. bassiana* were found superior over their commercial formulations. Wider adaptability and virulency of native strains of these bioagents might be responsible for its better activity against these emerging pests.

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