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Botanical biopesticide combination concept—a viable option for pest management in organic farming

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Abstract

Background: Entomopathogens are frequent natural enemies of arthropods worldwide, and they are capable of alternative control agents against the important pests. The optimally selected botanical product can minimize their harmful effect on these entomopathogens, and it becomes essential to know the influence of combinations of botanicals and biopesticides (botanical biopesticide combination (BBC)) in comparison to their sole action.

Main body: Botanicals, especially neem products, are highly efficient to be combined with the entomopathogens (with some exceptions). There are many possible reasons for the synergistic action of these botanicals, attacking the immune system of the insect being one of the important ones. These botanicals when applied in combination with microbial pesticides showed maximum sublethal effects rather than complete mortality, making them the best alternatives for combating resistance development in insects. To work effectively, biological control agents must be used within a compatible program combined with botanicals. It is highly difficult for such products to compete with chemical controls in high-value crops, so where they can become a commercially viable option in organic cultivation. The increasing acreage is under organic production for high-value export crops, where pesticide residues are undesirable for the environment, and biopesticides and botanicals are good choices for crop protection. Concerning the effect of these products used in pest control, a significant reduction in dosage in relation to the individuals is noticed.

Conclusion: By combining the performance and safety, biopesticides and botanicals are efficacious. This knowledge should facilitate the choice of biopesticides compatible with less harmful or naturally occurring botanicals. And if these have to be incorporated into a pest management program through an organic approach, it is necessary to determine the effects of botanicals on the beneficial microbes, on the behavior of pest, the importance of application technique, and the role of application timing for these botanical biopesticide combinations.

Keywords: Entomopathogens, Botanicals, Synergism, Combinations, Neem, Organic agriculture

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Background

The use of toxic chemicals for pest management has created many unwanted effects. The greatest problem is due to the residues of chemical insecticides and the development of resistance to these by the pests. Constant innovations are the only way to achieve success in the field of agriculture to cope with these biotic stresses. Due to the continuous evolution of these organisms leading to pesticide resistance, a continuous parallel research is needed to check them.

Since the 1800s, the wide-spread and increasing trend of chemical insecticide usage leading to many environmental imbalances and human health hazards. Farmers need to be switched to more effective and environmentally sustainable solutions of crop protection. Chemicals may be a dominant market today, but the fastest growing biological inputs are the future. The biological method provides the best alternative in maintaining the insect populations in balance without causing harm to other organisms and beneficial fauna in the ecosystem (Lv et al. 2011). In addition, bringing a chemical pesticide into the market needs a tiresome process with lots of effort and time, but the biopesticides can be commercialized in a year or so providing a good opportunity to fill the pest management gap created by chemicals.

Main text

Among the biological methods, botanicals play a major role as they are easily and locally available, and having ease of manufacture, they provide employment at the rural level. Botanicals had been used since the Vedic period for pest control (Koul and Walia 2009). Over three lakh plant species are estimated to have pesticidal properties but only 2400 species have been identified and still a lot more to be exploited (Thacker 2002). Only 10% of the compounds present in the array of plant chemicals are identified, indicating a huge future scope for research. Among these botanicals, neem is the top scorer because of its insecticidal compound, azadirachtin, and the limonoids present that are insecticidal to over 250 agricultural pest species (Morgan 2009). Researchers proved that neem (oil) is as effective as cypermethrin in controlling the tomato fruit borer, *Helicoverpa armigera* (Hb.) on tomato (Phukon et al. 2014). Neem products can be readily combined with many other bioagents including microbes (Mohan et al. 2007). Even eucalyptus contains many terpenoids like α and β pinene, 1,8-cineole (CIN), terpineol, and globulol (Lucia et al. 2012), which are found to have antifungal, antimicrobial, and insecticidal activities (both contact and fumigant) against many pests (Mann and Kaufman 2012). A number of other plants, namely *Derris elliptica*, *Nicotiana tabacum*, and *Acorus calamus* (Wongtang and Nawani 2001), and medicinal plants viz., *Euphorbia* sp., *Dodone*

avescosa, and *Chinus tribinlitolia* (Isman 2006), were found to have insecticidal properties.

Fungi with entomopathogenic effect can be readily incorporated in IPM tactics (Reddy et al. 2013) and more than 750 fungi from over 90 species are being entomopathogenic in nature (Zare and Gams 2001). They are the main pathogens of insects, causing about 80% of insect diseases (Alves et al. 2008). They enter through the integument and can infect all stages of insects, and if favorable conditions occur, they can create disease outbreaks in insects, especially Hemiptera, Coleoptera, and Lepidoptera (Shubakov and Kucheryavykh 2004). *Beauveria*, *Metarhizium*, *Verticilium*, *Nomurea*, *Aschersonia*, *Hirsutella*, and *Entomophthora* are those among commercially available genera (Alves et al. 2008). In the total number of entomopathogenic fungi (EPF) formulations developed worldwide, *M. anisopliae* and *B. bassiana* both share 33.9% each, followed by *Verticilium* spp. (9.4%), *Isaria fumosorosea* (5.8%), and *B. brongniartii* (4.1%). Among the type of formulations, substrates colonized by fungi are mostly available (26% of total formulation), and others include wettable powders (20.5%) and oil-dispersions (15.2%) (Etheimine et al. 2013). Entomopathogenic bacteria are the other most successful microbes after fungi used in pest control where particularly *Bacillus thuringiensis* (*Bt*) plays a king role with 2% of the insecticidal market (Bravo et al. 2011). The baculoviruses (nuclear polyhedral virus and granulosis virus) are one among insecticidal viruses pathogenic to insects, and they are specific to Lepidoptera and Diptera. They are successfully used in the management of universal pests like *Helicoverpa* sp. and *Spodoptera* sp. in different parts of the world (Rosell et al. 2008).

A couple of major exploited genera of entomopathogenic nematodes are *Steinernema* and *Heterorhabditis* (Sharifi et al. 2014), hosting *Xenorhabdus* and *Photorhabdus*, symbiotic bacteria, respectively, which are the primary cause for insect mortality within 24–72 h (Adams and Nguyen 2002). These two genera are capable of controlling a wide variety of insect pests of agriculture and forestry (Kulkarni et al. 2017; Paunika and Kulkarni 2019a, 2019b, 2019c). The only drawback with these microbes is that they are slow in action, and the problem can be tackled by incorporating them with other chemicals in different strategies. Recent approaches proved that “dual-attack” approach can result in a higher mortality of pest than their individual effect. The mixtures of two products are often more effective, showing the effect more their 1+1 effect, which is technically called synergism. Antagonism is precisely an opposite phenomenon in which the toxicity of two compounds is applied together is less than the expected sum of two individual effects. The less-discussed phenomenon is that antagonism is also likely to occur but mostly masked by positive

effects produced. Synergy and antagonism are indicated by many terms like co-toxicity coefficient, synergistic ratio, percent mortality, and many sublethal effects on pests reducing the yield loss. The combination of a botanical or a plant extract with insecticidal activity and an entomopathogen is a novel approach to fight against the resistances and resurgence issues created by insect pests (Srivastava et al. 2011). These botanical biopesticide combinations (BBC) are a boon to organic agriculture, resulting in an effective management, not less than a synthetic insecticide.

Synergism between botanicals and entomopathogenic bacteria

The early research on synergism reported that *Bt* when applied sequentially (Devi et al. 1996) or in combination (Trisyono and Whalon 1999) with neem products showed synergism against different agricultural pests. Synergistic results obtained in studies conducted at CRS,

Tirupati are shown in Fig. 1. *Btk* (*Bt* var *kurstaki* Serotype H-3a,3b; 3000 IU/mg, 9×10^9 viable spores/g, 5–8% endotoxin) and neem seed kernel extract (NSKE, when applied after 2 days of *Bt* spray) against *Achaea janata* (Linnaeus) on castor produced the highest yield, and the maximum mortality rate was also observed by *Bt* (0.25%) + NSKE (2%), where feeding cessation was observed immediately but mortality was not immediate after spray (Devi et al. 1996). Similarly, the commercial product of *Bt*, Delfin[®], was synergistic with neem extract but not with others, Spicturin[®] and Agree[®], and probably the action is due to additives in the former formulation. Even the sublethal concentrations of neem (0.25–0.45 mg a.i/L) combined with *Bt* (0.74 mg a.i/L) showed an additive effect on larval mortality of Colorado potato beetle, *Lepidotarsa decimlineata* (Trisyono and Whalon 1999).

The synergistic effects of neem products on *Btk* were proved by many research (Togbe et al. 2014; Konecka et al. 2019). The LC_{50} of *Bt* and azadirachtin (Singh et al.

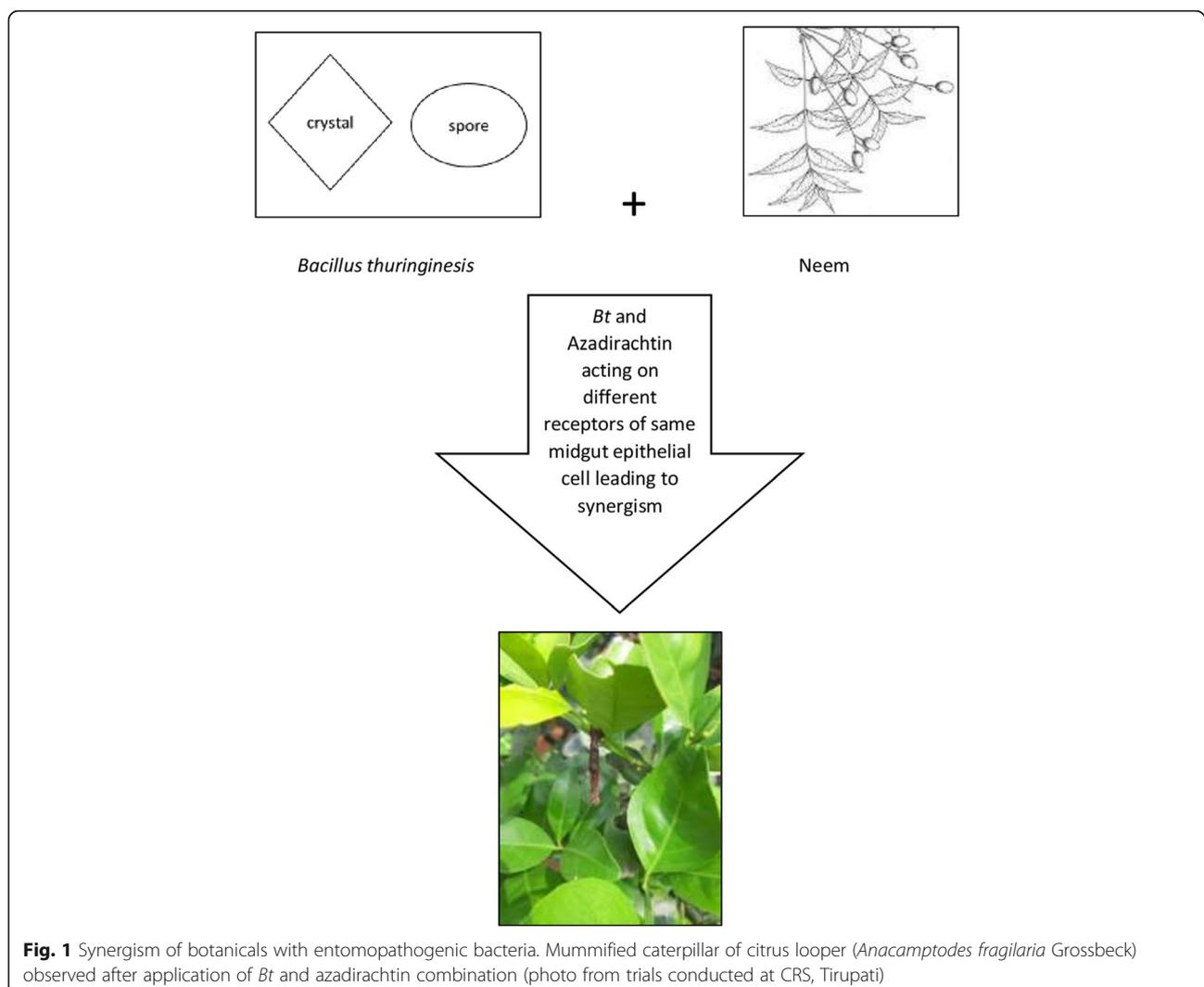


Fig. 1 Synergism of botanicals with entomopathogenic bacteria. Mummified caterpillar of citrus looper (*Anacamptodes fragilaria* Grossbeck) observed after application of *Bt* and azadirachtin combination (photo from trials conducted at CRS, Tirupati)

2007) and LC₂₀ of azadirachtin 0.09% EC (6.99 µg a.i./mL) and *Bt* 12.74% EC (51.62 µg a.i./mL) (Abedi et al. 2014) caused 100 and 56.7% mortality of *H. armigera*, respectively, showing a high level of synergism. This is also proved by Nouri-Ganbalani et al. (2016) that LC₅₀ concentrations of *Bt* (490 µg a.i./mL) and neem (241 µg a.i./mL) when combined showed synergistic effects, whereas the LC₃₀ combination showed additive interactions against *Plodia interpunctella*.

Apart from neem, there are some other botanicals with pest control properties, and one of them is palmarosa oil (*Cymbopogon martini* Wats.) (1%), which showed a synergistic activity with three *Bt* products, Delfin®, Spicturin®, and Agree®, against *Spodoptera litura* and *H. armigera*. Delfin® is found to be synergistic with extracts of neem, mentha, and *Prosopis juliflora* but not with Spicturin® and Agree®. This may be due to additives present in the formulation (Venkadasubramanian and David 1999). The extract of *Vitex pseudonegundo* (Hausskn) (Verbenaceae) was synergistic with *Bt* (Mancebo et al. 2002). Other botanicals, namely, *Annona squamosa* L., *Datura stramonium* L., *Eucalyptus globulus* (Labile), *Ipomea carnea* Jacq., *Lantana camara* L., *Nicotiana tabacum* L., and *Pongamia pinnata* (L.), all were synergistic with *Btk*, where *D. stramonium* being more synergistic with 74.7% mean larval mortality of *S. litura* (Rajguru et al. 2011). *Rumex tingitanus* was emphasized as a pest control agent for the first time (Mhalla et al. 2018) and was found to be larvicidal for 1st and 2nd instars and an antifeedant for 4th instar larvae of *S. litura*. In the nine different combinations of rumex extract with *Bt* strain BLB 250 (30 mg/g of diet), when tested against *S. litura* larva, seven of them showed synergism causing 100% mortality. Mustard oil can also be used along with *Bt* crystals for achieving the synergism against *Dendrolimus pini*, which resulted in twofolds higher mortality (Konecka et al. 2019). Recently, carvacrol, a monoterpenoid phenol, extracted

from aromatic plants (oregano) also shown synergistic effects with *Bt* against *Cydia pomonella* when mixed in the ratio of 1:25,000 and 1:50,000 (*Bt*: carvacrol) causing 1.5 and 1.9 times higher mortality, respectively. The ratio of 1:1000 and 1:2000 also showed 1.5- and 1.8-folds higher mortality of *Spodoptera exigua* than expected (Konecka et al. 2020). Some of the successful synergistic interactions between botanicals and EPB are shown in Table 1.

A few studies have found that botanicals also show antagonism with *Bt* like sweet flag (*Acorus calamus*), Indian aloe (*Aloe vera*), and bracteated birthwort (*Aristolochia bracteata*) against *S. litura* (Venkadasubramanian and David 1999), and *Acacia arabica* have demonstrated this activity against *S. litura* (Rajguru et al. 2011). Halder et al. (2017) observed that *Btk* mixed with neem oil showed incompatible interaction in controlling Epilachna beetle, *Epilachna deodecastigma*.

Synergism between botanicals and entomopathogenic fungi

The compatibilities of different strains of *Beauveria bassiana* with different neem products were proved by many researchers (Mohan et al. 2007; Islam et al. 2010; Sahayaraj et al. 2011; Avery et al. 2013). The synergism or compatibility of botanicals, especially neem products with EPF, was proved effective against *S. litura* (Mohan et al. 2007), a locust *Anacridum melanorhadon* (Haroon et al. 2011), *Bemisia tabaci* (Islam and Omar 2012), mealybugs (*Phenacoccus soleneopsis*) (Halder et al. 2013), and *Epilachna deodecastigma* (Halder et al. 2017). Eucalyptus extract was also proved to be viable for combining with *B. bassiana* against the wheat aphid, *Sitobion avenae* (Ali et al. 2018). It caused a high mortality rate and higher reduction of fecundity of the aphid *S. avenae*, than *B. bassiana* alone, *M. anisopliae*, and their combinations with neem and eucalyptus extract. Eucalyptus was thought to act on the nervous system, digestion,

Table 1 Few botanicals and entomopathogenic bacteria combinations

Combination	Pest targeted	Effect	Source
<i>Bt</i> (0.5%) + NSKE (5%) (sequential application within 2 days)	<i>A. janata</i> on castor	99.36% decrease in larval population after 5 days of sprays and showed the highest yield of 366.53 kg/ha	Devi et al. (1996)
<i>Bt</i> (0.25%) + NSKE (2%) (sequential application within 2 days)		99.58% decrease in larval population after 5 days of spray with a yield of 296 kg/ha	
<i>Bt</i> (Delfin) (0.1%) + palmarosa oil (0.5%)	<i>S. litura</i> <i>H. armigera</i>	96.29% mortality 96.48% mortality	Venkadasubramanian and David (1999)
<i>D. stramonium</i> seed extract fortified with <i>Btk</i>	<i>S. litura</i>	Highest mean larval mortality – 74.70%	Rajguru et al. (2011)
<i>Btk</i> (0.044%) + Azadirachtin (0.031%)	<i>Spilarctia obliqua</i> (in laboratory conditions)	70% mortality observed	Bandyopadhyay et al. (2014)
<i>B. thuringiensis</i> (BLB250) delta-endotoxins + <i>R. tingitanus</i> hexane extract (RtHexF) at 5 mg g ⁻¹	<i>S. littoralis</i>	100% larval mortality of 1st and 2nd instar larvae	Mhalla et al. (2018)

growth retardation, and reproductive ability and resulted in mortality of insect which had already penetrated by *B. bassiana* (Russo et al. 2015). The extracts of *Citrus vulgaris* with *B. bassiana* also showed synergism ratio of 1.20, representing a viable combination against third instar larvae of *Ephestia kuehniella* Zeller, where the LC_{50} value reduced to 92.32 from 110.98 when *B. bassiana* alone was used (Shakarami et al. 2015).

Metarhizium anisopliae was also considered to be synergistic when used in combination with botanicals viz. neem (Shoukat et al. 2016), pyrethrum (Fernández-Grandon et al. 2020), and 1-chlorooctadecane (Hussain and AlJabr 2020). The combination of pyrethrum and *M. anisopliae* (1×10^8 CFU/mL) reduced the *Aphis fabae* survival by 29.2 h, representing their synergistic activity (Fernández-Grandon et al. 2020). 1-Chlorooctadecane, a chemical obtained from the plant extracts of *Albertisia papuana* Becc, *Syzygium cumini* (L.), and *Arisaema amurense* Maxim, mixed with *M. anisopliae* (EBCL 02049) for controlling the date palm dust mite, *Oligonychus afrasiaticus*, also showed highly synergistic interaction with joint toxicity of 713 (also called co-toxicity coefficient, which is the sum of individual toxicity indices) (Hussain and AlJabr 2020).

Verticillium muscarium, another EPF (0.16 mg/l), and matrine, a quinolizidine alkaloid derived from the roots of *Sophora falvescens* and *S. alopecuroides* (0.83 mg/L), showed synergistic interaction against *B. tabaci* (Ali et al. 2017). The co-toxicity of these two compounds was found to be ranging 125.99 to 200.00, showing a fair level of synergism. Another nematocidal fungus *Paecilomyces lilacinus* was also found to be synergistic with botanicals namely neem cake and *Tagetes erecta*, and when applied to soil, the combination was effective against *Meloidogyne incognita*, reducing the root gall

index from 5 to 1 (Sundraraju and Kiruthika 2009). Each of the three EPFs, *B. bassiana*, *M. anisopliae*, and *L. lecanii* in 1:1 combination with neem oil, was synergistic which was well recorded in terms of co-toxicity coefficients ranging from 1.003 to 1.332 against *Epilachna dodecastigma* and *Bagrada hilaris* (Halder et al. 2017). *M. anisopliae* (IIVR strain) in combination with neem oil was most effective against the Hadda beetle (*E. dodecastigma*), and *L. lecanii* showed a promising mixture with neem oil against the painted bug (*B. hilaris*) (Halder et al. 2017). Some synergistic results of EPF with botanicals are given in Table 2.

Several studies have reported that neem products also have an inhibitory effect on these EPF (Rogerio et al. 2005) by suppressing the fungal growth and spore germination (Castiglioni et al. 2003). The surprising effect of fungi *M. anisopliae* when used alone repelled the parasitoid, *Aphidius colemani* of *Aphis fabae*, but by combining with pyrethrum, it had no negative effect on the parasitoid (Fernández-Grandon et al. 2020).

Synergism between botanicals and entomopathogenic virus

Helicoverpa armigera NPV (HaNPV) was one of the most compatible entomopathogenic viruses with botanicals, neem (Kumari 2012), *Tagetes patula*, and *Calotropis gigantean* (Rabindra et al. 1994). HaNPV was incorporated in the diet of *H. armigera* along with 10% aqueous leaf extracts of *T. patula* and *C. gigantea*. These botanicals activated latent infections and caused many sublethal effects, representing synergism (Rabindra et al. 1995). Again, when the commercial neem formulation (Econeem) was used in the mixture with HaNPV, it also showed a synergistic action by reducing the damage of

Table 2 Some botanicals and entomopathogenic fungi combinations

Combination	Pest targeted	Effect	Source
<i>P. fumosoroseus</i> + azadirachtin	Whitefly (laboratory bioassay)	20% extra nymphal mortality is observed, but azadirachtin has an inhibitory effect on fungi	James (2003)
<i>B. bassiana</i> + Neemix (4.5% azadirachtin)	<i>Tribolium castaneum</i>	Delays pupation but reduces mortality of pest	Akbar et al. (2005)
<i>P. lilacinus</i> + neem cake/ <i>Tagetes erecta</i> extract	<i>Meloidogyne incognita</i>	Reduced root gall index from 5 to 1	Sundraraju and Kiruthika (2009)
<i>B. bassiana</i> + <i>Citrus vulgaris</i> essential oil	<i>Ephestia kuehniella</i>	Highest synergism ratio of 1:20	Shakarami et al. (2015)
<i>L. muscarium</i> (0.8×10^8 conidia/mL) + matrine (0.75 mg/L)	<i>Bemisia tabaci</i>	98.31% mortality	Ali et al. (2017)
<i>B. bassiana</i> + eucalyptus extract	<i>Sitobion avenae</i>	Mortality rate (87%), the lowest fecundity (7 nymphs per female) was recorded	Ali et al. (2018)
<i>M. anisopliae</i> (1×10^6 CFU mL ⁻¹ or 1×10^8 CFU mL ⁻¹) + pyrethrum (10 ppm)	Bean aphid, <i>Aphis fabae</i>	At 1×10^6 CFU mL ⁻¹ survival was reduced by 29.2 h through addition of pyrethrum (from 67.3 h to 35.3 h), and by 10 h at 1×10^8 CFU mL ⁻¹	Fernández-Grandon et al. (2020)

Table 3 Synergism of insect virus and in combination with botanicals

Combination	Pest targeted	Effect	Source
<i>Spl</i> iNPV + Neemazal-T	<i>S. litura</i>	Increased the infectivity of <i>Spl</i> iNPV by 2.9-fold	El-Salamouny et al. (1997)
<i>S</i> /NPV 10 ⁴ PIBs/mL + NSKE (0.25%) + neem oil (0.25%)	<i>S. litura</i>	100% mortality of all 5 stages of larvae and also adult	Murugan et al. (1999)
<i>S</i> /NPV + neem oil/NSKE	<i>S. litura</i>	Reduced LC ₅₀ value of <i>S</i> /NPV by 1.05 to 1.43/1.03 to 1.33-folds	Muralibaskaran et al. (1999)
<i>Agse</i> GV + neem @ 10 ppm	<i>A. ipsilon</i> neonate larvae	Decrease of the LC ₅₀ value from 3.59 × 10 ⁷ in the virus alone to 7.11 × 10 ⁶ capsules/mL	Elnagar et al. (2004)
<i>Ha</i> NPV at 10 ³ PIB/mL + azadirachtin at 0.1 ppm	<i>H. armigera</i>	LT ₁₀₀ for 3rd instar larvae reduced to 72 h (for NPV and AZA, it was 168 and 120 h, respectively).	Kumar et al. (2008)
<i>Pb</i> GV + petroleum-ether extract of neem seed kernel (NSK)	<i>P. brassicae</i>	Maximum reduction of LC ₅₀ value (4.39 × 10 ² occlusion bodies [OBs] mL ⁻¹) as compared with <i>Pb</i> GV alone (1.85 × 10 ⁴ OBs mL ⁻¹)	Bhandari et al. (2009)
<i>Ha</i> NPV at 500 LE/ha + Neemarin (0.15%) at 700 mL/ha	<i>H. armigera</i> larva on tomato	86.41% mortality of larva	Kumari (2012)

Helicoverpa damage in cotton (71.99%) (Lingappa et al. 2000). The mortality of *H. armigera* was higher and LT₁₀₀ for 3rd instar larvae reduced from 120 to 168 h to about 72 h, when the same combination (Azadirachtin at 0.1% + NPV 10³ PIB/mL) was used (Kumar et al. 2008). Though endosulfan was found to be more synergistic and compatible with *Ha*NPV, from an ecological and organic agriculture point of view, neem seems to be a superior option in combination with NPV. The combinations *Ha*NPV at 250 and 500 LE/ha with Neemarin (0.15%) at 700 mL/ha caused 82.39 and 86.41% mortality, respectively (Kumari 2012).

Spodoptera litura NPV (*S*/NPV), another promising microbial pesticide, was also found to be synergistic with neem (Nathan and Kalaivani 2006). It was observed that LC₅₀ of *S*/NPV reduced from 1.43- to 1.05-folds by addition of neem oil and from 1.03- to 1.33-folds by addition of NSKE. The same case was with Nathan and Kalaivani's (2006) studies where *Spl*iNPV + azadirachtin at two different combinations, 1 × 10³ OB + 0.25 ppm and 1 × 10⁶ OB + 0.5 ppm, were found to be more synergistic and *S. litura* larvae died faster in the combination.

Granulosis virus (GV) of different agricultural pests can also be combined with botanicals to get some positive interaction effects (Bhandari et al. 2009). *Pieris brassicae* GV (*Pb*GV) was synergistic with botanicals in the order, NSKE > *Eupatorium adenophorum* > *Artemisia brevifolia* showing that neem is best compatible with *Pb*GV, and this particular combination recorded the lowest LC₅₀ value of 1.32 × 10³ OB/mL (Bhandari et al. 2009). Neem also has its positive effects on *Agrotis segatum* GV (*Agse*GV); particularly, neem oil and *Agse*GV combination decreased the LC₅₀ value from 3.59 × 10⁷ to 7.11 × 10⁶ capsules/mL diet on *A. segatum* (Elnagar et al. 2004). Synergistic effects of botanicals on EPV are shown in Table 3.

The findings of many research showed a positive synergism of botanicals with entomopathogenic viruses,

except for Cook et al. (1997) and Elnagar et al. (2004). It was noticed that there was no enhancement of viral activity when azadirachtin was added to *Lymantria dispar* NPV formulation (Cook et al. 1997). The one disadvantage might be of adding azadirachtin to a viral formulation is less virus production and release into the environment (Elnagar et al. 2004).

Synergism between botanicals and entomopathogenic nematodes

Numerous studies have recorded that entomopathogenic nematodes (EPN) appear to be compatible with herbicides, nematicides (Georgis and Kaya 1998), azadirachtin (Stark 1996), and *Bt* (Kaya et al. 1995) and also found to be synergistic with them (Koppenhöfer and Grewal 2005). The compatibility of botanicals to EPNs was recorded by many workers, viz., Neemarin to *Steinernema masoodi*, *S. seemae*, *S. carpocapsae*, *S. mushtaqi* (Rashid and Ali 2012), Neem suraksha to two species of *Steinernema* and three of *Heterorhabditis* (Hussaini et al. 2001), neem to *S. feltiae* (Krishnayya and Grewal 2002), neem to *S. carpocapsae* (Koppenhöfer and Grewal 2005; Kulkarni et al. 2009), neem and Nimor to *Heterorhabditis indica* (Sankar et al. 2009), neem to four nematode species, viz., *S. feltiae*, *S. asiaticum*, *H. bacteriophora*, and *H. indica* (Raheel et al. 2017). Similarly, other than neem, *Pongamia glabra*, *P. pinnata*, and other botanical combinations with *Agropest*, *Bt*, Biopahar, Ozomite, to the native species of India, *S. dharanaii* (Paunikar and Kulkarni 2020). The compatibility may differ with species, strains, doses, and adjuvants used in formulations (Koppenhöfer and Grewal 2005; Javed et al. 2008; Shamseldean et al. 2013); for example, *H. bacteriophora* was found to be more sensitive to plant extracts than *H. indica* (Shamseldean et al. 2013) and *S. feltiae* was more susceptible to Margosan-O (a formulation of neem seed extract) than *S. carpocapsae* and *S. glaseri* (Javed et al. 2008).

The different combinations of each neem product (NSKE, NeemAzal-T 5%, Neemix 4.5%) with *S. feltiae* resulted in 18 synergistic responses with NSKE, 19 with NeemAzal, and 11 with Neemix (Mahmoud 2007). The survival rates of *S. carpocapsae* combined with botanicals, viz., neem, tobacco, *Derris elliptica*, and *Acorus calamus*, was more than 94.5%, but the virulence of the nematode was decreased by longer soaking periods against *Galleria mellonella* larva by sand column assay (Nitjarunkul et al. 2015). In contrast, NeemAzal-U on *H. bacteriophora* though caused significant mortality of the nematode, but virulence was not affected (Meyer et al. 2012). Some successful synergistic interactions between botanicals and EPN are shown in Table 4.

Even though insecticides (chlorpyrifos/quinolphos at 0.04/0.05%) showed the highest *H. armigera* larval reduction (41.08%), the sequential application of *H. indica* + *Pongamia pinnata* (1 lakh IJs/L + 2.5%) or their combination (1.5 lakh IJs/L + 0.75 mL/L) recorded the same level of reduction, 37.15 and 36.92, respectively (Prabhuraj et al. 2005). The emerging rate and survival of nematodes was highest in camphor oil mixed with *H. indica* or *H. bacteriophora*, followed by garlic mixture with a nematode (Shamseldean et al. 2013). The other medicinal plant extracts, for instance, *Euphorbia pulcherrima* (0.714%) or garlic essential oil (0.067%) with *S. carpocapsae* or *H. bacteriophora* (500 IJs/mL), increased the pest mortality, affecting development, reproduction, and lifecycle under semi-field conditions against acridid grasshopper, *Heteracris littoralis* (Sharaby et al. 2013).

Though there are several reports on compatibility of neem formulations with EPNs, Meyer et al. (2012) revealed the negative effects of commercial neem formulations on nematodes. The soap surfactant in commercial neem products caused 23–25% mortality of *S. feltiae* (Krishnayya and Grewal 2002). In contrast, a slug parasitic nematode *Phasmarhabditis hermaphrodita* was more susceptible (mortality) with crude neem leaf extract than the commercial formulation, NeemAzal (Petricovszki et al. 2019), which might be due to the presence of high amounts of azadirachtin, triterpenes,

viz., nimbin, nimbidine, nimbinin, azadirachtol, salanin, and other such derivatives which are toxic to nematodes (Mondal and Mondal 2012). There was a variation in response of EPNs to neem-based products where it took 24 h for 100% mortality of *Galleria* larvae for *H. indica* alone, whereas along with neem, formulation (Nimor) took 48 h for 100% mortality, displaying the drawbacks of neem with EPNs (Sankar et al. 2009).

The infective juveniles of these EPNs were found to tolerate most of the pest control compounds for 2 to 6 h, and in this scenario, they can be tank-mixed before use and applied, without any loss of survival and virulence (Koppenhöfer and Grewal 2005). The advantage of using these combinations is botanicals being instant in action and the nematodes may become established for offering a long-term pest reduction (Klein and Georgis 1992).

Possible reasons for synergism

Insect immune system is a major target for botanical and microbial pesticide combinations (Konecka et al. 2020). The fitness of a plant extract for combining with a microbial insecticide depends on qualitative and quantitative variations of secondary metabolites, which might affect the microbes (Ribeiro et al. 2012). It shows a clear explanation that azadirachtin can induce significant cytotoxic effects on midgut epithelium, a primary target for *Bt* toxins. It shows that the reported synergism is due to *Bt* and azadirachtin binding to different receptors on the same midgut cell, leading to an absolute damage of the midgut epithelium (Roel et al. 2010). The synergism of neem and *Btk* may be due to the direct effect on enzyme regulation of the insect larvae (Bandyopadhyay et al. 2014). Carvacol, a plant-based extract from many aromatic plants, acted earlier (inhibitory effect on acetylcholinesterase activity), before the rupture of midgut cells by *Bt* (Konecka et al. 2020). Azadirachtin causes several effects on insects, viz., growth disruption, feeding, oviposition disruption, reduction in fitness, and fecundity, thus making it more susceptible to microbial infection (Defago et al. 2011). A hypothesized reason for synergism caused by neem products on EPF that the

Table 4 Some botanicals and entomopathogenic nematode combinations

Combination	Pest targeted	Effect	Source
<i>P. pinnata</i> (0.75 mL/L) + <i>H. indica</i> (1.5 lakh IJs/L)	<i>H. armigera</i>	36.92% larval reduction (insecticide – 41.08%)	Prabhuraj et al. (2005)
NSK (0.6%) + <i>S. feltiae</i> (800 IJs)	3rd instar larvae of <i>Bactrocera zonata</i>	76% mortality with the highest level of synergism	Mahmoud (2007)
NeemAzal T 5% (1.25%) + <i>S. feltiae</i> (100 IJs)	3rd instar larvae of <i>Bactrocera zonata</i>	100% mortality with a significant level of synergism	Mahmoud (2007)
<i>M.anisopliae</i> + NeemAzal-T + <i>S. carpocapsae</i>	<i>Frankliniella occidentalis</i> on French bean	32.7% deviation from additivity and showed a high level of synergism	Otieno et al. (2017)

growth retardation due to azadirachtin causes elongation of the inter-molt period, thus enabling more time for the fungus to attack the cuticle (Akbar et al. 2005). The compatibility of *B. bassiana* with insecticidal plant extracts is probably due to the variation in concentrations of phytoalexins, sulfurades, terpenoids, and triterpenoids (Depieri et al. 2005). Genetic variability of fungal strains is also the reason for differential compatibility to different phytosanitary products (Mohan et al. 2007). The rapid breakdown of pyrethrum and slow activation of EPF, if combined, the effect will be optimized compensating individual shortcomings (Fernández-Grandon et al. 2020). It was also observed that the reduction in *Agrotis segatum* larval weight is due to the antifeeding effect of neem and its effect on digestive enzyme activity and biochemical composition in the midgut, prior to the attack by a virus (Elnagar et al. 2004). The sublethal concentrations of individual components used in virus combinations do not cause the pest mortality directly but may induce changes in physiological behavioral activity (pest fitness) due to the combined botanical effect as they mainly act as stressors and show latent infection activators of EPV (Rabindra et al. 1995). Thus, the botanicals alter the pathophysiology of the insect during the subsequent viral attack (Nathan and Kalaivani 2006). The explanatory reason for synergism between botanicals and EPNs is less studied, and one such case is azadirachtin within 48 h of application completely stopped the development of larvae (*Otiorynchus sulcatus*), showing growth disruptive properties (Gaffney et al. 2005), and this may be the cause of attack by EPNs (Sanakar et al. 2009).

Conclusion

In most of the reviewed cases, the combinations of botanicals with the entomopathogenic agents showed certain levels of regulations of pests' populations. The combinations can create a new outstanding commercial bioinsecticide formulation giving boost to organic farmers, and the efficacy of these bio-combi products is especially to overcome the individual shortcomings of each product. Still, there is a dire need and scope for further studies on the effect on the behavior of pests, the importance of application technique, and the role of application timing for these botanical biopesticide combinations.

Abbreviations

Bt: *Bacillus thuringiensis*; LC₅₀: Lethal concentration for 50% of population; NSKE: Neem seed kernel extract; PIB: Poly inclusion bodies

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