A frontier area of research on liquid biopesticides: the way forward for sustainable agriculture in India

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Green revolution in India has immensely contributed in enhancing the crop productivity but not always with sustainable production. As excessive use of chemical pesticides is deleterious to the environment, microorganisms as biopesticides is emerging as a potential alternative for eco-friendly and sustainable plant protection. Carrier based solid biopesticide formulations have already proved their efficacy equivalent to chemicals. Rectifying the disadvantages of the solid formulations, liquid biopesticide formulations are emerging as the way forward for cost effective, eco-friendly and sustainable agriculture.

Agricultural intensification to feed an ever-growing population has resulted in the excessive use of synthetic chemical pesticides for reducing an estimated 45% crop losses due to pests, diseases and nematodes, amounting to Rs 290 billion per annum[1]. However, increasing public awareness on organic food owing to the negative effects of these chemicals on human health and environment like pollution, pesticide residues, pest resistance and resurgence has forced the researchers and pesticide industries to shift their focus to more reliable, sustainable and environment-friendly agents – biopesticides. These are the formulated forms of active ingredients based on microorganisms such as bacteria, viruses, fungi, nematodes or naturally occurring substances. They also include plant extracts and semiochemicals as insect pheromones[2].

Biopesticides have overwhelming advantages of high selectivity to target pests, safety to humans and non-target organisms, amenability to individual applications, integrated pest management and suitability for organic niche products, including export-oriented commodities. There are about 1400 biopesticide products sold worldwide, which is expected to produce a global market of US$ 3.2 billion by 2014 (ref. 3). In India, 16 biopesticides have been registered under the Insecticides Act, 1968 (ref. 4). The biopesticide market represents 2.89% of the overall pesticide market in India and it is expected to increase annually at a rate of about 2.3% in the future[5].

Despite several advantages of biopesticides, the rate of their consumption is not to the optimum level in comparison with the chemical pesticides mainly due to short shelf life, susceptibility to environmental conditions, expensive production systems and efficacy problems. This situation can be circumvented through formulation improvements and the liquid formulation technology is a promising alternative to overcome the drawbacks of conventional solid formulations of biopesticides. Currently, biopesticides are formulated mainly in solid carriers like talc, peat, lignite, clay, etc. However, these solid formulations suffer from major setbacks like shorter shelf life, high contamination and low field performance[6]. In addition, when talc formulations are used for management of plant diseases in horticultural and plantation crops through micro-irrigation techniques, concerns are raised regarding the blockage of nozzles and distribution of bioinoculants. Moreover, the process of biohardening in horticultural plantlets, tissue culture plants and hydroponics systems necessitates development of liquid-based bioformulations[7]. Liquid formulations offer longer shelf life (up to 2 years), with high purity, carrier-free activity, ease in handling and application, convenience in storage and transport, easy quality control and enhanced export potential[8]. In addition, they are also compatible with machinery on large farms and are preferred by farmers and industries.

Liquid biopesticides

Liquid biopesticide formulations are microbial cultures or suspensions amended with substances to improve stickiness, stabilization, surfactant and dispersal abilities[9]. These formulations use broth culture or liquid suspension mainly in water, and also in mineral or organic oils. They include nutrients, cell protectants and inducers responsible for cell/spore/cyst formation to improve efficacy[10]. Liquid biopesticides are usually formulated as emulsions, suspension concentrates, oil dispersions, suspo-emulsions, capsule suspensions and ultra-low volume formulations[11,12].

Additives in biopesticides

Researchers have successfully used common additives like glycerol, PVP (polyvinylpyrrolidone), CMC (carboxy methyl cellulose), gum arabic, sucrose, trehalose, etc. in liquid formulations to protect the cells from desiccation, osmotic pressure, temperature stress and drying[7,13,14]. In liquid formulation, the microbes are present in dormant cyst form which gives rise to active cells upon application in the field, and this helps increase the shelf life of liquid bioformuation for more than one year[15].

When the effect of different solvents or additives in liquid formulations was evaluated to maintain the population level of *Pseudomonas fluorescens*, it was found that glycerol (10 mM) recorded 9.50×10^7 cfu ml^{-1} up to 180th day, whereas PVP (2%) recorded 2.15×10^4 cfu ml^{-1} up to 120th day and declined thereafter[7]. Also glycerol, when added as a stabilizer in liquid formulations of *Bacillus thuringiensis* (*Bt*), recorded higher persistence of *Bt* cells at room temperature to control *Helicoverpa armigera* compared to solid formulations which were effective for one month[16].

Effect of liquid biopesticides on pathogenic fungi

Liquid formulations of *Pseudomonas fluorescens* Pf1, a bacterial biopesticide maintained high population of the bioagent

Table 1. Bio-efficacy of *Bacillus subtilis* 1% A.S. (liquid formulation) against disease complex of tomato

Treatment	Root knot index (1–5)		No. of *Meloidogyns incognita*/ 10 g of root		Disease incidence (%)		Yield/plot of 4×2.5 m (kg)		Increase in yield* (%)		Root colonization of *B. subtilis* (CFU/g of root) $\times 10^6$	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T_1	4.2	4.4	24	27	29.6	31.6	21.9	23.8	02.3	01.7	1.3	1.5
T_2	3.7	3.4	20	22	26.9	27.8	22.4	24.3	04.6	03.4	2.4	2.6
T_3	3.0	2.9	17	16	21.7	23.3	24.2	27.2	13.1	15.8	3.8	3.5
T_4	2.3	2.5	08	09	12.2	14.5	25.4	28.0	18.7	19.2	4.2	4.0
T_5	4.5	4.4	27	29	30.3	31.6	22.3	24.2	04.2	03.1	0	0
T_6	2.6	2.8	12	16	19.7	22.5	23.7	26.3	10.7	12.6	0	0
T_7	4.7	4.9	30	33	31.2	33.6	21.4	23.5	0	0	0	0
CD @ 5%	0.23	0.27	2.16	2.41	2.68	2.92	1.29	1.25	2.43	2.76	0.27	0.31

*CD was computed using angular transformed values.

T_1, Seed treatment with *B. subtilis* 1% A.S. – 20 ml/kg of seed; T_2, T_1 + nursery bed treatment with 50 ml *B. subtilis* 1% A.S.; T_3, T_2 + 2 tonnes vermicompost enriched with 2.5 l *B. subtilis* 1% A.S./ha; T_4, T_2 + 2 tonnes vermicompost enriched with 5.0 l *B. subtilis* 1% A.S./ha; T_5, 2 tonnes vermicompost/ha; T_6, Chemical treatment (carbofuran 1.0 kg a.i./ha + streptocycline 1 kg/ha); T_7, Control.

(10^7–10^8 cfu/ml) for up to 6 months, whereas talc-based formulations were effective for 3 months. This formulation, when used as seed treatment, seedling dip and soil drenching in tomato recorded minimum disease incidence of *Fusarium* wilt on tomato and increased fruit yield[7]. A liquid formulation of fungal biocontrol agent, *Trichoderma asperellum* added with starch as food base and small amounts of copper remained active for 6 months at room temperature and effectively colonized *Fusarium graminearum*[17].

Effect of liquid biopesticides on insect pests

Liquid formulations of *Pochonia* (=*Verticillium*) *lecanii*, in combination with adjuvants like glycerol, Tween 80 and arachnid oil were effective against the grapes mealy bug, *Maconellicoccus hirsutus* and recorded maximum surface coverage and biomass[18]. Bentonite oil-based liquid formulations (bentonite, corn oil, gum, glycerin) of entomopathogenic fungi, *Beauveria bassiana* were more effective than powder formulations in causing larval mortality of *H. armigera*, retaining spore viability for a longer time (6 months) and also ease in application[19]. An invert emulsion (water in oil) formulation of *M. anisopliae* was found effective against whiteflies, *Bemisia tabaci* and red spider mites, *Tetranychus cinnabarinus* in eggplants, and conidia remained viable for 30.8 months[20].

Effect of liquid biopesticides on nematode pests

Spray application of the bacterial suspensions of *Agrobacterium radiobacter* and *Bacillus sphaericus* isolates at densities of 9.7×10^8 and 3.16×10^9 cfu ml^{-1} respectively, caused significant reduction (24–41%) in root infection of potato by cyst nematodes, *Globodera pallida*[21]. Liquid formulations of *in vitro* produced endospores of bacterial bioagent, *Pasteuria penetrans* suppressed the host nematode, *Belanolaimus longicaudatus* more effectively (59–63%) than granular formulations (20–22%). The liquid formulation increased percentage of endospore attachment to nematode by 147–158 (ref. 22). The Indian Institute of Horticultural Research, Bengaluru has done pioneering work in biopesticides and has successfully commercialized many biopesticide technologies disseminated to more than 300 companies. Liquid biopesticides of *B. subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Trichoderma viride* and *Trichoderma harzianum* with shelf life more than 12 months have proved their efficacy on major nematode pests like root knot nematodes (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*) and lesion nematodes (*Radopholus similis*) affecting major horticultural crops like banana, papaya, carrot, capsicum, tomato, gerbera, carnation, etc. both in field and protected cultivation.

Liquid versus solid biopesticide formulations

When the effectiveness of *P. fluorescens* in solid and liquid formulations was compared against *Fusarium* wilt of tomato, liquid formulation of Pf1 recorded 81.21% control of the wilt disease while the talc-based formulation recorded 79.25% control under field conditions[7]. Comparison of liquid *B. subtilis* 1% A.S. formulation and talc-based solid formulation of *B. subtilis* 1% W.P. against root knot disease complex of tomato revealed better performance of liquid over solid formulations in reducing the root knot nematode population (55.5% higher) and disease incidence (20.33% higher) and increasing the yield (7.3% higher; Tables 1 and 2)[23,24].

Due to environmental concerns, the requirement for safer products in plant protection has created a huge demand for biopesticides with good efficacy and stability. This necessitated the development of liquid biopesticide formulations with more shelf life and efficacy than carrier-based solid biopesticide formulations. Liquid formulation is a budding technology in India and has specific characteristics and uniqueness. Liquid formulations are preferred by the farmers due to their ease in application and compatibility with modern irrigation systems, while in the case of solid formulations, there are difficulties like clogging of nozzles and uniform distribution of bio-inoculants[7]. It is a promising input to combat the problem

Table 2. Bio-efficacy of *B. subtilis* 1% W.P. (talc formulation) against disease complex of tomato

Treatment	Root knot index (1–5)		No. of *Meloidogyns incognita*/ 10 g of root		Disease incidence (%)		Yield/plot of 4 × 2.5 m (kg)		Increase in yield* (%)		Root colonization of *B. subtilis* (CFU/g of root) × 10^6	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T_1	4.3	4.6	25	24	31.5	30.2	25.0	23.0	01.8	01.5	1.5	1.7
T_2	4.0	3.9	23	22	28.3	29.1	25.1	23.2	02.4	02.2	2.1	2.2
T_3	3.3	3.2	19	16	24.2	23.2	27.1	25.9	10.2	11.4	3.2	3.0
T_4	2.6	2.9	12	14	17.5	18.2	28.3	26.1	15.2	14.9	3.5	3.3
T_5	4.4	4.6	25	26	29.3	30.1	25.7	23.6	04.4	04.2	0	0
T_6	2.7	2.8	14	13	23.4	22.8	27.6	25.3	12.3	11.7	0	0
T_7	4.7	4.6	34	29	33.9	35.6	24.6	22.7	0	0	0	0
CD @ 5%	0.21	0.24	1.95	2.24	2.74	2.31	1.73	1.96	2.79	2.61	0.45	0.36

*CD was computed using angular transformed values.

T_1, Seed treatment with *B. subtilis* 1% A.S. – 20 ml/kg of seed; T_2, T_1 + nursery bed treatment with 50 ml *B. subtilis* 1% A.S.; T_3, T_2 + 2 tonnes vermicompost enriched with 2.5 l *B. subtilis* 1% A.S./ha; T_4, T_2 + 2 tonnes vermicompost enriched with 5.0 l *B. subtilis* 1% A.S./ha; T_5, 2 tonnes vermicompost/ha; T_6, Chemical treatment (Carbofuran 1.0 kg a.i./ha + streptocycline 1 kg/ha); T_7, Control.

of low viability of bioagents and satisfies the cost-effectiveness. In addition, the longer viability of beneficial microbes in liquid formulations reduces the frequency of application to crops and thereby decreases the cost of plant protection. Use of liquid formulations of microbial consortia against sunflower necrosis virus resulted in a cost benefit ratio of 1 : 6.8, whereas using chemicals, the ratio was 1 : 3.7 (ref. 25). Proper attention is needed towards the technology to manifest the results at field level by increasing awareness among farmers. Liquid biopesticides have the capacity to replace the traditional chemical pesticides and carrier-based solid biopesticides, and play a major role in crop protection for sustainable agriculture and food security. Further improvement of techniques and multidisciplinary research of plant protection scientists and agricultural engineers are likely to provide good, safe, effective and inexpensive biopesticide formulations for plant protection. Measures in terms of technology, government support, subsidies and constructive awareness among the agrarians are emphasized.

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