Biopesticide Formulation to Control Tomato Lepidopteran Pest Menace[1*]

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Abstract

In the present study an indigenous biopesticide formulation (BPF) comprising easily accessible botanicals along with cow urine, was evaluated for its efficacy against insect pests of tomato crop under field. BPF gave promising results in controlling tomato fruit borers and afforded substantial yield of the produce. The BPF treatment could control 70–80% of fruit borers compared to check plots, resulting in enhanced fruit yield of 35 tons ha^{-1} as compared to 15 tons ha^{-1} in the check plots. The main aim of this study was to reduce the load of synthetic chemical pesticides and evaluate indigenous knowledge as an alternative component of pest management to have pesticide residue-free tomato.

Tomato (*Lycopersicon esculentum*) is the world's second important vegetable crop known for its protective food because of its special nutritive value and its widespread production. In India nearly 7.1 million tons of tomato is produced annually, ranking it fifth in the world, from an area of 5.4 lakh ha, placing the country at the second position globally based on its area of production. On an average about 10,800 tons of tomato is exported annually from India. The major importers of Indian tomatoes are Bangladesh, Nepal, Pakistan, and the UAE (NAIP, 2007).

Because of its fleshy nature, tomato fruit is attacked by a number of insect pests and diseases (Pandey *et al.,* 2006; Reddy *et al.,* 2007), resulting in the consumption of large amounts of pesticides which leave their toxic residues (Kumari *et al.,* 2002). As it is a short-duration crop and gives high yield, it is important from an economic point of view. Spider mites (*Tetranychus urticae* Koch), whitefly (*Bemisia tabaci* Genn), leaf miner (*Liriomyza trifolii*), and borers (*Helicoverpa armigera* Hübner) are serious pests on tomato causing considerable yield loss under open field conditions

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in India (Reddy *et al.,* 2007). The yield loss in tomato crop due to fruit borer (*H. armigera*) alone amounts to 22–38% (Dhandapani *et al.,* 2003) or rupees one thousand crores per annum (Padmanaban and Arora, 2002). *Heliothis armigera* is a polyphagous pest and has been reported to infest 181 cultivated and uncultivated plant species in India (Manjunath *et al.,* 1989); it accounts for 90–95% of the total damage to the fruit commodity (Sithanantham *et al.,* 1983; Sachan and Katti, 1994). Synthetic chemicals may be used in plant protection programs to limit crop damage by pests and pathogens. But because of growing concerns about health and environmental safety, the use of toxic, carcinogenic and/ or environmentally damaging chemicals is being discouraged. A survey of monitoring the farm-gate samples in different parts of the country recorded pesticide residues above maximum residue limit (MRL) (Madan *et al.,* 1996; Chahal *et al.,* 1997; Kole *et al.,* 2002; Mandal and Singh, 2010).

The individual botanicals are not able to control crop pests, when the pest pressure is high or when there is epidemic in the field. But they can be effective as one of the components of either integrated pest management (IPM) or with other control measures for pest management. Unlike these botanicals, synthetic pesticides give instant action in controlling crop pests (exceptional cases for resistance development) along with their economical and easy access. Due to this, farmers and sometimes researchers relying on botanicals invariably discard them and switch to persistent and toxic synthetic pesticides. Therefore, a need was felt to have a reliable biopesticide formulation (BPF), which could be applied even at the time of an epidemic, when insect or disease population is high under field conditions. The BPF was prepared and tested for its efficacy in in-vitro as well as in-vivo studies. It was prepared by mixing nine natural ingredients of bio-botanical origin with one naturally occurring mineral salt along with one animal product, in specific ratios in a liquid (also animal product). These natural products, namely onion, ginger, *Ocimum*, neem, etc. are reported for their in-vitro efficacy (Chowdhury *et al.,* 2000; Jacques *et al.,* 2004; Ogechi and Marley, 2006; Slusarenko *et al.,* 2008), individually; but their efficacy at field level is not reported at par with synthetic or a combination of these ingredients (Sadawarte and Sarode, 1997; Hegde and Nandihalli, 2009). Under field conditions, they are not reported to manage pests if they cross the economic threshold level (ETL) and hence are not effective for pest control at the time of the epidemic.

This communication describes one such product prepared for pest management in tomato crop. The indigenously prepared biopesticide is environmentally sound, nature-friendly, and economical.

The BPF comprised of 12 ingredients; nine of them were bio-botanical in origin; two were natural mineral salts and one was an animal product (cow dung) respectively, all mixed in a liquid (cow urine) which is also an animal product. The indigenous cow breed was used for BPF. Fresh cow dung was taken in the morning and sieved through a muslin cloth to get its extract. The ratio and proportion of ingredients was standardized according to their economics and availability. Ratio of ingredients along with plant part used for preparing 1000 ml of BPF was standardized and is given in Table 1.

The raw material used for this formulation was mashed and mixed thoroughly in cow urine of indigenously bred cow in an earthen pot. The pot was then buried in soil for 30 days for fermentation. Then the contents of the pot were thoroughly mixed and the solution was considered as 100% stock solution.

In order to study the response of tomato crop to different treatments of BPF, field trials were conducted in randomized block design at the Indian Agricultural Research Institute (IARI), New Delhi, for two consecutive years during 2006–07 and 2007–08. Tomato crop (Pusa Hybrid-2) was raised in the farms of Division of Agronomy, IARI, during November, with six treatments, including control (in triplicate) and transplanted in the first week of February. The organic treatments were chosen deliberately for

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Table 1. Composition of biopesticide formulation.

Ingredients	Ratio of ingredients (%)
Phyllanthus emblica (*amla*) fruit	4
Curcuma zedoaria (turmeric)	6
Potassium aluminum sulfate dodecahydrate (naturally occurring mineral salt) [$KAl(SO_4)_2.12(H_2O)$]; alum (*phitkari*)	5
Allium cepa (onion) bulb	3.5
Allium sativum (garlic) bulb	4
Calotropis procera	5
Fresh cow dung extract (cow dung taken in morning)	3
Lycopersicon esculentum (tomato) leaf extract	6
Ferula narthex	2
Azadirachta indica leaves	5.5
Ocimum canum (*tulsi*) leaves	4
Cow urine	52

their comparative study with BPF treatment, so that BPF can be adopted as a component in organic farming of tomato crop. For organic treatment, a uniform application of vermicompost at 6 tons ha^{-1} was made in all the plots, 10 days prior to transplanting. Two sprays of neem oil at 3% were applied at flowering and fruiting stage in the organic treatments.

The five different treatments were: BPF at 5%; BPF at 5% + organic; BPF at 10%; BPF at 10% + organic; and organic. All the treatments were with zero input (no input of fertilizers was supplied to any of the treatments), and control with no treatment. The plot size was 3 × 3.6 m^2 with row-to-row and plant-to-plant distance of 50 cm. For BPF treatment, it was applied twice at 3% at the nursery stage followed by four sprays at 5% and 10%; one each at flowering and fruiting, and two during (at an interval of 20 days) the maturing stage. The crop was monitored for pest population by observing damaged fruit data after BPF spray in all six treatments. Each plant was monitored for insect borers and data were analyzed. The yield data were recorded for all the treatments.

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The BPF comprised of 12 ingredients; nine of them were bio-botanical in origin; two were natural mineral salts and one was an animal product (cow dung) respectively, all mixed in a liquid (cow urine) which is also an animal product.

The BPF containing cow urine and other easily accessible botanicals have been reported individually for their bioactivity, but their combined bioactivity, when these are mixed together has not been reported.

The fruit damage observed in 10% BPF-treated plots was only 3–4% compared to 35–40% in control plots and 16% in organically treated plots. It was slightly higher in 5% BPF (5–7% damage) and 10% BPF + organic (4–5% damage) treated plots. The damage observed was 8–11% in 5% BPF + organic plots. Because of least damage in BPF at 10% treated plots, the highest yield was observed. It was 35–36 tons ha^{-1} compared to 15 tons ha^{-1} in check plots and 17 tons ha^{-1} in organically treated plots. The data indicate that 10% BPF treatment was the best for controlling fruit borers of tomato crop.

The formulation tested against insect pests of tomato crop, using two sprays at nursery stage and four sprays at standing crop, was found promising in controlling tomato fruit borers resulting in good yield of the produce with zero input (Kanojia *et al.,* 2008). The organically treated plots could produce 170 q ha^{-1}, whereas the plots treated with BPF alone at 10% gave more than 350 q ha^{-1}

yield of tomato fruits, for both the years (Table 2). The control untreated plots gave the lowest yield with 145 q ha^{-1} only. Although yield of organic treatments was higher than that of control plots (no treatment), it was statistically far below the yield realized from BPF-treated plots. Treatments with BPF at 5% alone and along with organic treatments could also give better yield compared to organic treatments alone. But BPF at 10% was found to be the best treatment.

Table 2. Yield data (2006–08) of tomato crop for two consecutive years.[1]

Treatment	Fruits/ plant (g)	Marketable fruits		Damaged fruits (kg/plot)	Gross yield		Marketable mean yield for 2006–08 (q/ha)
		kg/plot	q/ha		kg/plot	q/ha	
Yield (2006–07)							
T1 Untreated (control)	600.2	16.4[a]	152.2	8.8	25.2	233.3	
T2 BPF at 5%	829.7	24.1[bc]	222.8	2	26	240.7	
T3 BPF at 5% + organic	782.3	24[bc]	222.2	1.4	25.4	235.2	
T4 BPF at 10%	1298	39.4[e]	364.4	1.4	40.8	377.8	
T5 BPF at 10% + organic	1220.7	36.2[de]	335.6	1.7	37.9	350.9	
T6 Organic (residual with zero input)	667.5	18.9[ab]	175.1	3.6	22.5	208.3	
Yield (2007–08)							
T1 Untreated (control)	572.7	15.4[a]	142.9	9.9	25.3	234.3	147.5
T2 BPF at 5%	909.3	25.6[c]	237.3	3.2	28.8	266.7	230
T3 BPF at 5% + organic	809	24.9[c]	230.4	2	26.9	249.1	226.3
T4 BPF at 10%	1237.7	38[de]	351.7	1.4	39.3	363.9	358
T5 BPF at 10% + organic	1162.7	33.5[d]	310.5	1.9	35.4	327.8	323
T6 Organic (residual with zero input)	668.2	18.6[ab]	172.3	3.6	22.3	206.5	173.7

1. CD calculated for marketable fruits/plot at 1% is 5.44 and at 5% is 3.614 (2006–07); 7.989 and 5.31 at 1% and 5% respectively for 2007–08. The treatments have been grouped using Duncan's method in SPSS statistical analysis for marketable fruits only. Data bearing the same letter(s) as superscript show no significant difference, whereas those with different letter(s) as superscript are significantly different from each other.

The raw material used for this formulation (of BPF) was mashed and mixed thoroughly in cow urine of indigenously bred cow in an earthen pot. The pot was then buried in soil for 30 days for fermentation. Then the contents of the pot were thoroughly mixed and the solution was considered as 100% stock solution.

The CD calculated for marketable fruits/plot for 2006–07 was 5.44 at 1% and 3.61 at 5% respectively. Similarly it was 7.99 at 1% and 5.31 at 5% for 2007–08 respectively. The statistical analysis of yield data showed that T1 and T6; T2 and T3; T4 and T5 are not significantly different from each other, whereas T4 is significantly different from the rest for both the years. The CD was also calculated for marketable yield data combined for both the years by taking all the treatments (treatments 1–6 for 2006–07 and 7–12 for 2007–08) in one run, so as to compare yield data of 2006–07 with 2007–08. Its value was 7.13 and 5.26 at 1% and 5% respectively. Based on CD analysis, different groups were formed (using Duncan's method in SPSS statistical package, version 17.0) for all the treatments for both the years. It is clear from the analysis that T1 and T6 are the same for both the years, while T2 and T3 are not significantly different in both years. T4 is not significantly different from T5 of 2006–07 and T4 of 2007–08, but significantly different from T5 of 2007–08. T4 was significantly different from T1, T2, T3, and T6. So, there is no change in yield data based on change in year (climate).

The promising results prompted us to perform the nutrient analysis of BPF, as it may have worked as plant growth regulator (Table 3). The report indicated that this formulation had good amount of macro- and micronutrients. As 4.36 ppm of zinc (Zn) indicates 4.36 mg in 1 L, therefore 200 L (required for 1 ha of land) of this formulation would contain 1 g of Zn as micronutrient. For micronutrients in sulfate form, 10–15 kg ha^{-1} each of Zn, Cu, Fe, Mn are required. So biopesticide is a bonus to plants in the form of nutrients besides controlling pests of the tomato field crop. Generally 20% of nutrients, applied to soil, gets absorbed by the plants, and the rest is not available to the plants. They get converted to insoluble or inorganic forms of nutrients. Table 4 shows the amount of micronutrients required for plants and the level below which it causes deficiency symptoms in the plants.

Phosphorus (P) is especially essential for early growth and root development, whereas nitrogen (N) and potassium (K) are fundamental in ensuring normal growth and production of quality fruit. Adequate K can enhance fruit quality by influencing sugar levels, as well as fruit ripening and storage characteristics. Soil K deficiency can lead to uneven, blotchy ripening, high levels

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of internal white tissue, yellow shoulder, decreased lycopene, and irregular shaped and hollow fruits. Tomato has a relatively high K requirement compared to N. Demand for K is highest during fruit bulking. About 2.6–3.6 kg of K is required for each 1000 kg of harvested tomato, as reported by Bose *et al.* (2006).

The US Patent 7297659 relates to a synergistic composition useful as plant and soil health enhancer, comprising urine, neem, and garlic, individually or in all possible combinations. It has the ability to stimulate accumulation of nutrients in the plant biomass, promote plant-growth, phosphate-solubilization, abiotic stress-tolerance, and antagonism towards plant pathogenic fungi, control phytopathogenic fungi in the rhizosphere of plants, and enhance the total phenolic contents of the plants.

Allicin from garlic effectively controlled seed-borne *Alternaria* spp. in carrot, Phytophthora leaf blight of tomato, and tuber blight of potato as well as *Magnaporthe* on

Table 3. Report of nutrient analysis of cow urine-based BPF formulation.

Nutrients	Available element in soluble ionic form (ppm)
Macronutrients	
N	37,900 (3.79%)
P	Could not be done
K	8250 (0.8%)
Micronutrients	
Zn	4.36
Cu	0.27
Fe	45.3
Mn	5.75
Mg	76.4
Other secondary nutrients	
Ca	60.9
Na	1631

Table 4. Amount of micronutrients available to plants.

Micronutrient	Amount (ppm)
Zn	1 (<0.6 ppm, it is said to be deficient in zinc)
Cu	0.5 (<0.2 ppm, it is said to be deficient in copper)
Fe	10–15 (<4.5 ppm, it is said to be deficient in iron)
Mn	5 (<2.0 ppm, it is said to be deficient in manganese)

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rice and downy mildew of *Arabidopsis* (Slusarenko *et al.,* 2008).

The effect of crude extracts of neem (*Azadirachta indica*) leaf, neem seed, and garlic (*Allium sativum*) at concentrations ranging from 5% to 30% of the material in 100 ml of potato dextrose agar on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was assessed. All the extracts inhibited mycelial growth at various levels. Dry neem seed extract gave 100% inhibition of mycelial growth (Ogechi and Marley, 2006).

Curcuminoids, the major coloring constituents of *Curcuma longa* (turmeric) rhizome powder, comprise mainly three closely related curcumins (I–III). These have been tested along with the parent compounds and other extractives for insect growth inhibitory activity against *Schistocerca gregaria* and *Dysdercus koenigii* nymphs. At 20 µg/nymph, benzene extract and dibutyl curcumin-I were most active (60% inhibition) against *S. gregaria*, whereas at 50 µg/nymph these substances exhibited moderate growth-inhibitory activity (45%) against *D. koenigii* nymphs (Chowdhury *et al.,* 2000).

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The biocidal properties of garlic, onion, and leek are attributed to sulfur volatiles produced during degradation of *Allium* tissues. The primary emitted compounds are thiosulfinates and zwiebelanes mainly converted in the soil or in *Allium* products (extracts) to disulfides. The activities of these compounds were studied in-vitro on soil pathogenic fungi and insects in order to measure their disinfection potential. These studies show a good potential for three disulfides: dimethyl disulfide, dipropyl disulfide, and diallyl disulfide to inhibit several fungal species (Jacques *et al.,* 2004). Reed (1939) reported that tomato plants grown in copper-deficient nutrient solutions showed characteristic dwarfing, involution of the leaflets, color change, and eventual necrosis. Based on its efficacy and promising results in pest control and yield of target crops, this BPF has been filed for patenting in India (Kanojia *et al.,* 2009).

The indigenous BPF was observed to give promising results in controlling tomato fruit borer along with good yield of the produce. India produces about 7.5 million tons of tomato from about 450,000 ha. Current average world yield stands at 27 tons ha^{-1}, while current average productivity in India is 17 tons ha^{-1}. But yield from indigenously treated tomato fields in this experiment was 32 tons ha^{-1}. Further studies need to be conducted for different growth parameters of tomato plant by making use of this BPF, as tomato fruit weight and yield are highly dependent on K rate and BPF acted like a plant growth promoter besides its role in pest control in plant protection. The technique may be validated against other crops, as it is economical, socially acceptable, leaves no toxic residues in the environment, uses easily accessible inputs and therefore can strengthen the national IPM programs. The broad spectrum, synthetic conventional pesticides affect the non-target organisms, therefore it should be ensured to include this kind of indigenous knowledge in pest management programs. Therefore such tactics must be readily accessible to agricultural researchers, development practitioners, and policy makers.

The field dosage (5% and 10%) was decided according to laboratory studies for tomato crop. It can be increased or decreased according to the target pest and field crop studied. The BPF may not give rise to phytotoxicity, because it has proved to be a nutrient supplier for plant growth. We did not test the BPF beyond 10%, but it may not be harmful for use beyond that value.

Biopesticides are effective in small quantities and decompose quickly when used as a component of IPM programs. They can greatly decrease the use of conventional pesticides, keeping crop yields high. With the use of conventional pesticides, a safe waiting period has to be followed according to the recommendations, but here the fruits can be consumed the same day after harvesting. As the fruits are perishable, this would be beneficial to the farmers.

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