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Biopesticide formulation to control tomato lepidopteran pest menace

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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 tonnes/ha as compared to 15 tonnes/ha 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 alternate component of pest management to have pesticide residue-free tomato.

Keywords: Biopesticide, cow urine, pest control, 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 tonnes 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 tonnes of tomato is exported annually from India. The major importers of Indian tomatoes are Bangladesh, Nepal, Pakistan and the UAE[1].

Because of its fleshy nature, tomato fruit is attacked by a number of insect pests and diseases[2,3], resulting in the consumption of large amounts of pesticides which leave their toxic residues[4]. 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* Hubner are serious pests on tomato causing considerable yield loss under open field conditions in India[3]. The yield loss in tomato crop due to fruit borer (*H. armigera*) alone amounts to 22–38% (ref. 5) or one thousand crores rupees per annum[6]. *H. armigera* is a polyphagous pest and has been reported to infest 181 cultivated and uncultivated plant species in India[7]; it accounts for 90–95% of the total damage to the fruit commodity[8,9]. Synthetic chemicals may be used in plant protection programmes to limit crop damage by

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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 farmgate samples in different parts of the country recorded pesticide residues above maximum residue limit (MRL)[10–13].

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[14–17], individually; but their efficacy at field level is not reported at par with synthetic or a combination of these ingredients[18–19]. 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 bio-pesticide is environmentally sound, nature-friendly and economical.

Table 1. Composition of biopesticide formulation

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

The BPF comprised of 12 ingredients; nine of them were of 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 used for BPF ingredients was normal, healthy and milking. 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 breed 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.

For *in vitro* bio-insecticide studies on *Pieris brassicae*, larvae of the insect were collected from *brassicae* fields and were reared on cabbage leaves. The third instar larvae were taken as test insects for bioassay studies. Five concentrations (0%, 1%, 3%, 5% and 10%) of this formulation were prepared by serial dilution of the crude formulation with distilled water. The bioassay studies were carried out using leaf dip method[20]. Ten 6-day-old, third instar larvae were released on each disc in an individual petri plate. For control sample, the leaf disks were dipped in distilled water for the same time. Four replications were used for each concentration, including control. Observations were recorded on larval mortality in each treatment at 12 and 24 h intervals up to 48 h after treatment.

The experiment was conducted for bioassay testing of *Spodoptera litura* under laboratory conditions for calculation of field dosage of BPF. The insect was reared on castor leaves. The culture of *S. litura* was maintained at 25 ± 2°C, 70 ± 5% relative humidity and 16 : 8 h photo : scotophase and maintained in laboratory on castor leaves (*Ricinus communis* L.) according to the methodology of Kamaraj *et al*.[21].

The experiment was performed with seven concentrations (0%, 1%, 2%, 5%, 10%, 12% and 15%) of BPF, each with five replicates, including control. Five 7-day-old, third instar larvae were released on treated leaves of castor plant put in a bottle covered with muslin cloth. For control sample, the leaves were dipped in distilled water for the same time. Observations were recorded as mentioned above.

In order to study the response of tomato crop to different treatments of BPF, the 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 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 @ 6 tonnes/ha was made in all the plots, 10 days prior to transplanting. Two sprays of neem oil @ 3% were applied at flowering and fruiting stage in the organic treatments.

The five different treatments were: BPF @ 5%; BPF @ 5% + organic; BPF @ 10%; BPF @ 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 sq. m with row-to-row and plant-to-plant distance of 50 cm. For BPF treatment, it was applied twice @ 3% at the nursery stage followed by four sprays @ 5% and 10%; one each at the flowering, fruiting and two in between (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 analysed. The yield data were recorded for all the treatments. Different weather parameters are listed in Figure 1, as recorded during tomato field crop for two years.

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.

In vitro bioefficacy studies were conducted on *P. brassicae* because of availability of its larvae. Although it is an insect pest of *brassicae* and not of tomato crop, it was also tested for *in vitro* bioefficacy of BPF. The larval mortality of *P. brassicae* was observed up to 48 h of exposure at regular intervals of treatment with BPF. Mean mortality of insect was very low for 1% and 3% dosage, but more than 50% for 5% concentration of BPF. More than 90% mortality of this insect was observed with 10% of the formulation (Table 2). LC_{50} calculated for BPF using EC_{50} calculator software was 4.7%.

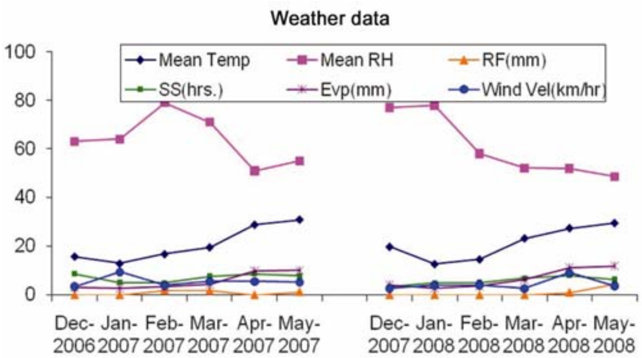

Figure 1. Weather data recorded during tomato field crop for two years.

The *in vitro* experiments were conducted to find the field dosage of BPF to be sprayed on the tomato crop. Although no significant mortality of insect larvae was observed under this test experiment, feed deterrent activity was observed for BPF-treated insect larvae, which varied in ascending order with increase in concentration of the biopesticide. After 72 h of treatment, the insects were provided with fresh untreated leaves for feeding, which were consumed up to 90% in 0% and 2% doses, whereas only 20% food was consumed for higher concentrations (10% and 15%). The antifeedant character of insect larvae was observed as residual activity in treated feed.

Statistical analysis of recorded fruit damage data is presented in Table 3. Each plant was observed for borers and data in Table 3 are an average of all 30 plants in a plot. The six treatments (T_1 – untreated-control, T_2 – BPF @ 5%, T_3 – BPF @ 5% + organic, T_4 – BPF @ 10%, T_5 – BPF @ 10% + organic and T_6-organic: residual with zero input) were replicated thrice under experiments for two consecutive years to test the effect of climate, if any, and reproducibility of the experiments. For statistical analysis, SAS package (version 9.2) was used. The analysis of variance was performed to find the effect of treatment, replicate and year. Table 3 shows significant difference among treatments according to $Pr > F$ as 0.0001 at 5% level of significance. It is clear from the table that change due to year is not significant. Reproducibility was observed among replicates of each treatment. Duncan multiple range test has also been applied for pairwise comparison of treatments. It is observed that T_1 and T_6 are significantly different. However T_2, T_5, T_3 and T_4 are significantly different from T_1 and T_6, but not significantly different from each other. The damage after applying T_4 is the least.

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 @10% treated plots, the highest yield was observed. It was 35–36 tonnes/ha compared to 15 tonnes/ha in check plots and 17 tonnes/ha 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 (Figure 2)[22]. The organically treated plots could produce 170 q/ha, whereas the plots treated with BPF alone @ 10% gave more than 350 q/ha yield of tomato fruits, for both the years (Table 4). The control untreated plots gave the lowest yield with 145 q/ha only. Although yield of organic treatments was higher than that of control plots (no treatments), it was statistically far below the

Table 2. *In vitro* bioefficacy of BPF against *Pieris brassicae*

Concentration (%)	Percentage mortality of larvae after 48 h					LC (%)	Fiducial limits	
	R_1	R_2	R_3	R_4	R_5		Minimum	Maximum
0.0	0	0	0	0	0	LC_{50} = 4.68	4.6	4.7
1.0	0	1	0	0	1	LC_{10} = 2.32	2.25	2.39
3.0	21	20	19	18	22	LC_{20} = 3.00	2.9	3.07
5.0	57	56.5	55	53	53.5			
10.0	92	91	90	92.5	93			

LC_{50} value for this formulation is 4.68%.

Table 3. Statistical analysis for tomato fruit damage data

Source	DF	Sum of squares	Mean square	*F* value	*Pr* > *F*
Treatment	5	491.0138889	98.2027778	192.88	<0.0001
Replicates	2	0.5659722	0.2829861	0.56	0.5800
Year	1	0.0625000	0.0625000	0.12	0.7288
Error	27	13.7465278	0.5091307		
Corrected total	35	505.3888889			

Duncan's multiple range test for damage

Alpha	0.05
Error degrees of freedom	27
Error mean square	0.509131
Critical value of *t*	2.05183
Least significant difference	0.8453

Treatment comparison	Difference between means	95% confidence limits		
T_1–T_6	6.0000	5.1547	6.8453	***
T_1–T_2	9.5000	8.6547	10.3453	***
T_1–T_5	9.7083	8.8631	10.5536	***
T_1–T_3	10.0000	9.1547	10.8453	***
T_1–T_4	10.3750	9.5297	11.2203	***
T_6–T_1	–6.0000	–6.8453	–5.1547	***
T_6–T_2	3.5000	2.6547	4.3453	***
T_6–T_5	3.7083	2.8631	4.5536	***
T_6–T_3	4.0000	3.1547	4.8453	***
T_6–T_4	4.3750	3.5297	5.2203	***
T_2–T_1	–9.5000	–10.3453	–8.6547	***
T_2–T_6	–3.5000	–4.3453	–2.6547	***
T_2–T_5	0.2083	–0.6369	1.0536	
T_2–T_3	0.5000	–0.3453	1.3453	
T_2–T_4	0.8750	0.0297	1.7203	***
T_5–T_1	–9.7083	–10.5536	–8.8631	***
T_5–T_6	–3.7083	–4.5536	–2.8631	***
T_5–T_2	–0.2083	–1.0536	0.6369	
T_5–T_3	0.2917	–0.5536	1.1369	
T_5–T_4	0.6667	–0.1786	1.5119	
T_3–T_1	–10.0000	–10.8453	–9.1547	***
T_3–T_6	–4.0000	–4.8453	–3.1547	***
T_3–T_2	–0.5000	–1.3453	0.3453	
T_3–T_5	–0.2917	–1.1369	0.5536	
T_3–T_4	0.3750	–0.4703	1.2203	
T_4–T_1	–10.3750	–11.2203	–9.5297	***
T_4–T_6	–4.3750	–5.2203	–3.5297	***
T_4–T_2	–0.8750	–1.7203	–0.0297	***
T_4–T_5	–0.6667	–1.5119	0.1786	
T_4–T_3	–0.3750	–1.2203	0.4703	

Trials conducted at experimental plots with zero input. Comparisons significant at the 0.05 level are indicated by ***.

Figure 2. Effective visibility of the indigenous biopesticide formulation (BPF) with zero inputs.

Table 4. Yield data (2006–2008) of tomato crop for two consecutive years

Treatment	Yield (2006–07)						Yield (2007–08)						Marketable mean yield for 2006–2008 (q/ha)
	Fruits/ plant (g)	Marketable fruits kg/plot	Marketable fruits q/ha	Damaged fruits (kg/plot)	Gross yield kg/plot	Gross yield q/ha	Fruits/ plant (g)	Marketable fruits kg/plot	Marketable fruits q/ha	Damaged fruits kg/plot	Gross yield kg/plot	Gross yield q/ha	
T_1 Untreated (control)	600.2	16.4^{a}	152.2	8.8	25.2	233.3	572.7	15.4^{a}	142.9	9.9	25.3	234.3	147.5
T_2 BPF @ 5%	829.7	24.1^{bc}	222.8	2	26	240.7	909.3	25.6^{c}	237.3	3.2	28.8	266.7	230
T_3 BPF@ 5% + organic	782.3	24^{bc}	222.2	1.4	25.4	235.2	809	24.9^{c}	230.4	2	26.9	249.1	226.3
T_4 BPF @ 10%	1298	39.4^{e}	364.4	1.4	40.8	377.8	1237.7	38^{de}	351.7	1.4	39.3	363.9	358
T_5 BPF@ 10% + organic	1220.7	36.2^{de}	335.6	1.7	37.9	350.9	1162.7	33.5^{d}	310.5	1.9	35.4	327.8	323
T_6 Organic (residual with zero input)	667.5	18.9^{ab}	175.1	3.6	22.5	208.3	668.2	18.6^{ab}	172.3	3.6	22.3	206.5	173.7

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

yield realized from BPF-treated plots. Treatments with BPF 5% alone and along with organic treatments could also give better yield compared to organic treatments alone. But BPF @ 10% was found to be the best treatment.

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 T_1 and T_6; T_2 and T_3; T_4 and T_5 are not significantly different from each other, whereas T_4 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 T_1 and T_6 are the same for both the years, while T_2 and T_3 are not significantly different in both years. T_4 is not significantly different from T_5 of 2006–07 and T_4 of 2007–08, but significantly different from T_5 of 2007–08. T_4 was significantly different from T_1, T_2, T_3 and T_6. 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 5). The report indicated that this formulation had good amount of macro- and micronutrients. As 4.36 ppm of zinc indicates 4.36 mg in 1 l, therefore 200 l (required for 1 ha of land) of this formulation would contain 1 g of zinc as micronutrient. For micronutrients in sulphate form, 10–15 kg/ha 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, the rest is not available to the plants. They get converted to insoluble or inorganic forms of nutrients. Table 6 shows the amount of much 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 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.*[23].

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.

Table 5. 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 6. Amount of micronutrients available to plants

Zn	1.00 ppm (< 0.6 ppm, it is said to be deficient in zinc)
Cu	0.5 ppm (< 0.2 ppm, it is said to be deficient in copper)
Fe	10–15 ppm (< 4.5 ppm, it is said to be deficient in iron)
Mn	5.00 ppm (< 2.0 ppm, it is said to be deficient in manganese)

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 rice and downy mildew of Arabidopsis[14].

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 mycellial growth at various levels. Dry neem seed extract gavel 100% inhibition of mycelial growth[15].

Curcuminoids, the major colouring 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[16].

The biocidal properties of garlic, onion and leek are attributed to sulphur volatiles produced during degradation of *Allium* tissues. The primary emitted compounds are thiosulphinates and zwiebelanes mainly converted in the soil or in *Allium* products (extracts) to disulphides. 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 disulphides: dimethyl disulphide, dipropyl disulphide and diallyl disulphide to inhibit several fungal species[17]. Reed[24] reported that tomato plants grown in copper-deficient nutrient solutions showed characteristic dwarfing, involution of the leaflets, colour 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[25].

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 tonnes of tomato from about 450,000 ha. Current average world yield stands at 27 tonnes/ha, while current average productivity in India is 17 tonnes/ha. But yield from indigenously treated tomato fields in this experiment was 32 tonnes/ha. 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 programmes. 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 programmes. 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 programmes. 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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