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Evaluation of seed biopriming with biocontrol agents and biopesticides spraying on pests and its effect on seed yield and quality in chilli

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Abstract
The field experiment was conducted to find out the effect of seed biopriming with biocontrol agents using *Trichoderma viride* 60% for 9h and *Pseudomonas fluorescens* 60% for 12 h along with nonprimed seed, which constituted the main plot treatments in Field No. 37E of TNAU, Coimbatore exclusively maintained for organic cultivation situated in North Western agro-climatic zone of Tamil Nadu during *Kharif* 2013 and *Rabi* 2013. Biopesticide sprays of Panchakavya 3%, dashparni extract 10%, neemastra 10%, agneyastra 3%, brahmastra 5%, cow's urine 5%, neem seed kernel extract 5% and neem oil 1% along with imidacloprid 0.3 ml/l formed the subplot treatments. For organic seed production, the results revealed that, seed biopriming with *P. fluorescens* 60% for 12 h and neem seed kernel extract 5% sprays were highly effective in both the seasons.

Keywords: Chilli seeds, *Trichoderma viride*, *Pseudomonas fluorescens*, biopesticide

Introduction
Important limiting factor in the cultivation of chilli is insect pests and diseases. The insects such as, the sucking pests like aphid, thrips, mite is known to cause damage to the crop [30]. To tackle the pest menace, a number of chemical insecticides are liberally sprayed on this vegetable crop which leads to several problems like toxic residues, elimination of natural enemies, environmental disharmony and development of resistance [14]. Due to the presence of pesticidal residues in the commodity, there is also a risk of rejection of whole consignments during export. To overcome these problems, identification of safe molecules with better insecticidal properties, lower mammalian toxicity, safe to natural enemies etc., which fits well in the IPM concept, is the need of the hour [26]. Plant kingdom is a vast store house of bioactive chemicals which are isolated and tested for insect controlling properties *viz.,* repellent, anti-feedant, hormonal and insecticidal activity [26]. At present, for the management of diseases in vegetable crops, chemical fungicides are the first choice for the farmers [8]. With increasing awareness of possible deleterious effects of fungicides on the ecosystem and growing interest in pesticide free agricultural products, biological control now appears to be a promising strategy for managing diseases in a range of crops [8].
Biological factors such as fungi and bacteria are used in biopriming and the most important of all are *Trichoderma viride* and *Pseudomonas fluorescence* [34]. Application of *Trichoderma* and *Pseudomonas* in agriculture has several beneficial effects such as plant root and its rhizosphere colonization and competence, control of soil and plant pathogens by parasitism, antibiotics production and promotion of systemic resistance which ultimately improves plant health through increasing root and plant growth [5, 17].
Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management [9]. Botanical pesticides are ecofriendly, economic, target-specific and biodegradable. Keeping the above research gaps in view, the present investigations were made by conducting field studies with chilli cv. PKM 1 with the objectives of suitable biopesticides control pests and its effect on seed yield and quality.

Materials and methods
Pure and fresh seeds of chilli (PKM 1) obtained from the Department of Seed Science and Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore formed the base material for this study. The biocontrol agents *Trichoderma viride* (28×106 cfu/g) and *Pseudomonas fluorescens* (2.5 × 106 cfu/g) were collected from the Department of Plant

Pathology, TNAU, Coimbatore. The biopesticides namely dashparni extract, neemastra, agneyastra, brahmastra, neem seed kernel extract and panchakavya were prepared in the laboratory of Seed Science and Technology were used. The field experiment were conducted in Field No. 37E of TNAU, Coimbatore exclusively maintained for organic cultivation situated in North Western agro-climatic zone of Tamil Nadu at 11ºN latitude and 77º longitude with an altitude of 426.72 msl during *Kharif* 2013 and *Rabi* 2013.

To determined the effect of seed biopriming, biocontrol agents *Trichoderma viride* 60% for 9 h (M_2), *Pseudomonas fluorescens* 60% for 12 h (M_3) along with nonprimed seed (M_1) were used [1]. The biopesticides, panchakavya 3% (S_2), dashparni extract 10% (S_3), neemastra 10% (S_4), agneyastra 3% (S_5), brahmastra 5% (S_6), cow's urine 5% (S_7), neem seed kernel extract 5% (S_8) and neem oil 1% (S_9) were used along with imidacloprid 0.3 ml/l of water (S_1). The experiment was laid out in split plot design during two seasons *Kharif* and *Rabi* 2013. The biopriming treatments formed the main plot treatments and biopesticides spraying formed the subplot treatments. The observations recorded for applications of biopesticides on crop growth parameters, yield and yield components, pests and disease incidence, population of natural enemies, yield and quality of seed. The data obtained from various experiments were analysed statistically adopting the procedure described by [21].Wherever necessary and the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance (*). If F test is non-significant, it was indicated as NS.

Results and Discussion

In the present study, plant height (66.8 and 64.2 cm), leaf area index (1.672 and 1.659) and chlorophyll index (45.4 and 44.0) were more in the plants grown from the bioprimed seed with *P. fluorescens* and imidacloprid spray than the control in plant height (63.0 and 62.4 cm), leaf area index (1.630 and 1.628) and chlorophyll index (43.4 and 42.9). Among the biopesticides spraying, seed bioprimed with *P. fluorescens* with neem seed kernel extract 5% spray recorded the plant height of 63.0 and 62.4 cm, leaf area index of 1.630 and 1.628 and chlorophyll index of 43.4 and 42.9 during both the seasons (Table 1 and 2). An improvement in growth parameters noticed in this study due to biopriming could be attributed to suppression of deleterious microorganisms and pathogens, production of plant growth regulators such as gibberellins, cytokinins and indole acetic acid, increased availability of minerals and other ions and extensive rooting which facilitates water and nutrient uptake [27]. Effectiveness of bio-priming with *P. fluorescens* in improvement of growth was evident from the initial stages over the control. Similar improvement of seed quality by rhizobacteria has been reported earlier in pearl millet [19, 21] and also in other cereals such as sorghum and rice [23]. Spraying of neem seed kernel extract 5% has improved the plant height in bhendi [14]. Higher leaf area index and chlorophyll index measured in this study due to neem seed kernel extract 5% spray is in good agreement with the results of [13] in rice and [22] in tomato. This might be attributed to the fact that neem seed kernel extract/neem seed cake contains high nitrogen content of 5.22%, more protein of about 28% and more sulphur than other cakes/kernels of plant origin [11] which might have contributed for increased plant growth and development.

Table 1: Effect of seed biopriming and biopesticides spraying on plant height (cm), leaf area index and chlorophyll index at 90 DAT in chilli cv. PKM 1 in *Kharif 2013*

Main plot / Subplot treatments		Plant height (cm)				Leaf area index				Chlorophyll index			
		M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Imidacloprid @ 0.3 ml/lit. (S_1)		63.0	64.9	66.8	64.9	1.630	1.655	1.672	1.652	43.7	44.9	45.4	44.7
Panchakavya 3% (S_2)		56.5	57.0	58.2	57.2	1.535	1.560	1.587	1.561	38.0	38.6	39.7	38.5
Dashparni extract 10% (S_3)		59.2	59.8	60.7	59.9	1.592	1.601	1.627	1.607	39.9	41.1	41.7	40.9
Neemastra 10% (S_4)		60.0	60.5	61.4	60.6	1.613	1.615	1.642	1.623	40.5	41.8	42.2	41.5
Agneyastra 3% (S_5)		58.4	58.9	59.9	59.1	1.579	1.595	1.611	1.595	39.2	40.5	41.5	40.4
Brahmastra 5% (S_6)		57.0	57.5	59.0	57.8	1.557	1.572	1.599	1.576	38.7	39.9	41.0	39.9
Cow's urine 5% (S_7)		55.2	56.8	57.0	56.3	1.529	1.553	1.575	1.552	37.3	37.8	39.2	37.9
NSKE 5% (S_8)		61.0	61.5	63.9	62.1	1.631	1.645	1.659	1.645	42.0	42.9	43.4	42.8
Neem oil 1% (S_9)		60.4	61.0	62.0	61.1	1.622	1.629	1.640	1.630	41.2	42.9	42.8	42.3
Mean		59.0	59.8	61.0		1.587	1.602	1.623		40.1	41.1	41.7	
	M	T	M x T	T x M	M	T	M x T		T x M	M	T	M x T	T x M
SEd	0.054	0.420	0.689	0.729	0.001	0.004	0.007		0.007	0.503	0.506	0.968	0.876
CD (P=0.05)	0.108	0.844	1.378	1.458	0.002	0.009	0.015		0.014	1.009	1.011	1.932	1.755

M_1- Nonprimed seed; M_2 - Biopriming with *T. viride* 60% for 9 h ; M_3 - Biopriming with *P. fluorescens* 60% for 12 h
DAT-Days after transplanting

Table 2: Effect of seed biopriming and biopesticides spraying on plant height (cm), leaf area index and chlorophyll index at 90 DAT in chilli cv. PKM 1 in *Rabi* 2013

Main plot / Subplot treatments		Plant height (cm)				Leaf area index				Chlorophyll index			
		M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Imidacloprid @ 0.3 ml/lit. (S_1)		62.4	63.1	64.2	63.2	1.630	1.642	1.659	1.644	42.9	43.4	44.0	43.4
Panchakavya 3% (S_2)		55.8	56.2	57.4	56.5	1.523	1.532	1.545	1.533	37.8	38.5	39.0	38.7
Dashparni extract 10% (S_3)		58.7	59.8	60.7	59.7	1.569	1.582	1.595	1.582	39.6	40.3	40.9	40.3
Neemastra 10% (S_4)		59.9	60.5	61.3	60.6	1.582	1.596	1.608	1.595	40.2	40.8	41.6	40.9
Agneyastra 3% (S_5)		58.0	59.1	59.0	58.7	1.559	1.575	1.582	1.572	39.0	39.7	40.5	39.7
Brahmastra 5% (S_6)		56.9	57.8	58.0	57.6	1.540	1.555	1.569	1.555	38.2	39.2	40.0	39.1
Cow's urine 5% (S_7)		54.6	55.7	56.0	55.4	1.507	1.519	1.523	1.516	37.1	38.0	38.5	38.1
NSKE 5% (S_8)		61.0	62.2	62.5	61.9	1.610	1.619	1.625	1.618	41.5	41.9	42.5	42.0
Neem oil 1% (S_9)		60.4	61.8	62.0	61.4	1.595	1.605	1.611	1.604	40.9	41.4	42.0	41.4
Mean		58.6	59.6	60.1		1.568	1.580	1.591		39.7	40.3	41.1	
	M	T	M x T	T x M	M	T	M x T	T x M	M	T	M x T	T x M	
SEd	0.054	0.420	0.689	0.729	0.002	0.003	0.006	0.006	0.503	0.506	0.968	0.876	
CD (P=0.05)	0.108	0.844	1.378	1.458	0.005	0.006	0.012	0.013	1.009	1.011	1.932	1.755	

M_1- Nonprimed seed; M_2 - Biopriming with *T. viride* 60% for 9 h ; M_3 - Biopriming with *P. fluorescens* 60% for 12 h
DAT-Days after transplanting

Vigorous growth in terms of plant height, leaf area index and chlorophyll index noticed in the plants grown from seeds bioprimed with *P. fluorescens* and foliar spray with imidacloprid and neem seed kernel extract 5% resulted in early flowering (28.0, 28.2 days and 28.5, 29.0 days) respectively during both the seasons. Phytotonic effect of imidacloprid increased growth parameters such as plant height, leaf area index, chlorophyll index and early flower initiation over control. Similar findings of advancement in flowering by 5 days were observed by [18] in pearl millet, seed treatment with *Pseudomonas* reached 50 per cent flowering a week earlier than other. [25] observed early flowering by 1.2 days in the plants which received neem seed kernel extract 5% spray.

For thrips population counted after four sprayings indicated that bioprimed seed with *P. fluorescens* along with imidacloprid as well as neem seed kernel extract 5% were found effective in controlling the thrips during both the seasons. Whereas, in all the four sprays, the least performing biopesticides was cow's urine. The period of residual effect with respect to insecticide and biopesticide was similar in all the sprayings. For mites population counted on the first and second spraying indicated that bioprimed seed with *P. fluorescens* along with imidacloprid as well as neem seed kernel extract 5% was also found effective in controlling the mites in both the seasons. Regarding the management of pests like thrips and mites, in chilli organic seed production, next to imidacloprid spray, spraying of neem seed kernel extract 5% was effective when compared to other biopesticides like panchakavya, dashparni, neemastra, agneyastra, brahmastra and cow's urine. Among several botanical biopesticides, neem based biopesticides are the most wanted biopesticides because they possess combination of the pest control properties like antifeedant, repellent, chemo sterilant, attractant, juvenile and anti-juvenile and anti-hormone, ovicide, nematicide, rodenticide, anti-viral, fungicide and bactericide. These multifaceted biological effects on pests enable neem products to control more than 200 different species of insects [6, 16]. According to [29], more than 450 insects species have been found to be susceptible to azadirachtin and exhibited various behavioural and physiological effects. Though azadirachtin, a triterpenoid, is present in all parts of the neem plant, it is abundantly present in the kernels (2-3 mg g^{-1} of kernel). The site of synthesis and accumulation have been identified in secretory cells which are the most abundant in seed kernels [31]. Primarily, it acts as antifeedant *i.e.,* when an insect larva is hungry and it wants to feed on the leaf but if the leaf is treated with neem products, because of azadirachtin, salanin and melandriol there is an antiperistalitic wave in the alimentary canal and this produces something similar to vomiting sensation in the insects. Because of this sensation, the insect does not feed on the neem treated surface and ability to swallow is also blocked. Secondly, it acts as ovipositor deterrent i.e., by not allowing the female to deposit eggs. Though imidacloprid was effective in controlling the most important destructive pests of chilli that causes severe damage to the plant, ultimately and higher loss of yield than biopesticides, it is harmful to beneficial insects and natural enemies of the crop. Therefore, it is better to use biopesticides like neem seed kernel extract 5% for effective control of these pests as well as to save the natural enemies [2].

The present study indicated that, seeds bioprimed with *P. fluorescens* 60% for 12h and the plants sprayed with neem seed kernel extract 5% protected the population of natural enemies like spider and coccinellid than imidacloprid spray. Many previous findings support the safety of neem based biopesticide formulation towards predators and parasitoids. [10] reported that the number of syrphid fly larvae in the field was not reduced after spraying of neem Azal-F on peach trees. Similarly neemix was found to have little or no impact on lady bird beetles, parasitic wasps, spiders and predatory mites [30]. The products had no toxicity on eggs, larvae, adults and fecundity of the predator. The neem products must be ingested to be effective, therefore insects, which feed on plant tissues, are affected by the extract and those which feed on other insects rarely contact lethal concentrations, which may lead to their insensitivity to the neem extracts. Similar results were reported by several research workers [15, 3]. In the present study, the use of imidacloprid was superior in controlling the pests of chilli, but it is not desirable to use synthetic insecticide due to many negative effects like pesticide residues, destruction of predators and parasites, environmental pollution, destabilization of ecosystem and enhanced resistance to insecticides in pests. So, there is a need to explore eco-friendly and cost-effective control methods. One such method is neem seed kernel extract 5%as observed in this study.

In this study, seeds bioprimed with *P fluorescens* and sprayed with imidacloprid produced more fruit weight per plant than nonprimed seeds and sprayed with cow's urine in both the seasons. Among organic biopesticides, neem seed kernel extract 5% recorded more fruit weight per plant in both the seasons (Table 3 and 4). The seed yield, its attributes and quality are highly depended mainly on the better growth and development of plants as well as free from pests and disease which can be achieved only by proper mother crop nutrition and appropriate plant protection measures. The probable reason for considerable enhancement in the seed yield and its attributing parameters might be due to the beneficial effects of biocontrol agents in protecting the pathogen attack on seed and crop, supply of growth promoting substances and mobilization of insoluble ions and nutrients from soil to the plant. Similar results were reported for many crops where seed treatments with *P. fluorescens* have increased growth parameters and other reproductive parameters [4].

Table 3: Effect of seed biopriming and biopesticides spraying on fruit set percentage (%), seed yield/ ha (kg) and 1000 seed weight (g) in organic seed production in chilli cv. PKM 1 in *Kharif 2013*

Main plot / Subplot treatments	Fruit set percentage (%)				Seed yield/ha (kg)				1000 seed weight (g)			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Imidacloprid @ 0.3 ml/lit. (S_1)	80.8	81.6	82.1	81.5	208.5	214.6	216.8	213.8	6.36	6.38	6.43	6.39
Panchakavya 3% (S_2)	73.5	74.3	75.1	74.2	180.0	185.5	191.5	185.7	5.61	5.79	5.87	5.76
Dashparni extract 10% (S_3)	77.0	78.1	78.6	77.7	193.5	196.5	200.5	196.8	5.93	6.03	6.13	6.03
Neemastra 10% (S_4)	77.9	78.4	79.5	78.4	196.0	199.6	201.3	199.0	6.00	6.10	6.20	6.10
Agneyastra 3% (S_5)	76.1	76.7	77.4	76.6	188.5	191.5	198.6	192.9	5.85	5.99	6.05	5.97
Brahmastra 5% (S_6)	75.7	76.1	76.5	76.1	185.0	188.9	194.5	189.5	5.78	5.89	5.93	5.87
Cow's urine 5% (S_7)	74.2	75.0	75.9	75.0	176.7	180.7	189.0	182.1	5.53	5.62	5.80	5.65
NSKE 5% (S_8)	79.7	80.0	80.6	80.1	203.2	206.0	210.0	205.9	6.19	6.26	6.32	6.26
Neem oil 1% (S_9)	78.9	79.1	79.8	79.1	199.5	203.4	205.9	202.9	6.07	6.19	6.27	6.18
Mean	77.1	77.7	78.1		192.5	196.3	200.7		5.92	6.03	6.11	

	M	T	M x T	T x M	M	T	M x T	T x M	M	T	M x T	T x M
SEd	0.374	0.534	0.950	0.926	0.146	0.882	1.448	1.528	1.196	2.300	3.942	3.984
CD (P=0.05)	0.748	1.069	1.905	1.857	0.292	1.765	2.896	3.051	2.395	4.601	7.889	7.865

M_1- Nonprimed seed; M_2 - Biopriming with *T. viride* 60% for 9 h ; M_3 - Biopriming with *P. fluorescens* 60% for 12 h

Table 4: Effect of seed biopriming and biopesticides spraying on fruit set percentage (%), seed yield/ ha (kg) and 1000 seed weight (g) in organic seed production in chilli cv. PKM 1 in *Rabi* 2013

Main plot / Subplot treatments	Fruit set percentage (%)				Seed yield/ha (kg)				1000 seed weight (g)			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Imidacloprid @ 0.3 ml/lit. (S_1)	78.0	80.6	81.1	79.9	207.7	209.0	211.5	209.7	6.42	6.48	6.51	6.47
Panchakavya 3% (S_2)	72.4	73.2	74.9	73.6	164.9	171.3	175.4	170.5	5.18	5.36	5.83	5.46
Dashparni extract 10% (S_3)	75.2	76.6	78.0	76.8	183.8	186.1	191.0	187.0	5.65	5.87	6.10	5.87
Neemastra 10% (S_4)	75.9	77.6	78.9	77.7	188.6	194.1	196.7	193.1	5.90	6.03	6.15	6.02
Agneyastra 3% (S_5)	74.1	75.3	77.1	75.6	176.5	180.4	186.9	181.3	5.52	5.73	6.04	5.77
Brahmastra 5% (S_6)	73.2	74.8	76.0	74.7	170.0	176.2	179.3	175.2	5.40	5.56	5.95	5.63
Cow's urine 5% (S_7)	71.0	71.8	74.5	72.4	160.8	166.0	168.2	165.0	5.03	5.20	5.78	5.33
NSKE 5% (S_8)	77.3	79.2	80.0	78.8	198.6	203.5	208.5	203.3	6.12	6.26	6.37	6.25
Neem oil 1% (S_9)	76.9	78.9	79.4	78.5	195.2	197.7	202.0	198.3	6.01	6.11	6.27	6.13
Mean	74.9	76.4	78.0		183.0	187.1	191.0		5.69	5.84	6.11	

	M	T	M x T	T x M	M	T	M x T	T x M	M	T	M x T	T x M
SEd	0.305	0.511	0.889	0.886	0.701	0.825	1.519	1.429	0.420	2.039	3.442	3.624
CD (P=0.05)	0.611	1.023	1.779	1.769	1.403	1.651	3.041	2.861	0.843	4.079	6.887	7.245

M_1- Nonprimed seed; M_2 - Biopriming with *T. viride* 60% for 9 h ; M_3 - Biopriming with *P. fluorescens* 60% for 12 h

The higher seed yield due to imidacloprid reported in this study is in close agreement with the findings of [25, 28] who also reported increased seed yield in bhendi. The second best treatment in this study which enhanced the organic seed yield was seed biopriming and spraying of neem seed kernel extract 5%. This finding is in good agreement with the findings of [25, 28] and they were also in view that spraying of neem seed kernel extract 5% improved the seed yield in bhendi. The relative increase in organic seed yield due to neem seed kernel extract 5% spray was not only because of enhanced growth, but also control of pests and diseases in bhendi as reported by several researchers [7, 12].

The quality of resultant organic seed harvested from the plants grown from the bioprimed seed with *P fluorescens* and sprayed with neem seed kernel extract 5% was higher during *Kharif* 2013 (78, 83, 89, 85 and 84 per cent) and during *Rabi* 2013 (77, 81, 86, 85 and 84 per cent) when compared to other treatments. The lowest germination per cent was recorded by nonprimed seed spraying with cow's urine in during *Kharif* 2013 (70, 76, 82, 80 and 78 per cent) and during *Rabi* 2013 (68, 71, 77, 77 and 75 per cent). Similar trend was followed for root length, shoot length, drymatter production and vigour index. The enhanced organic seed quality observed in this study might be due to the fact that the *P fluorescens* and neem seed kernel extract 5% had actively involved in the plant metabolism through supply of growth hormones, nutrient mobilization and efficient water uptake which resulted in increased carbohydrate metabolism and better accumulation of photosynthates in the seed. Similar improvement of seed quality parameters by rhizobacteria has been reported earlier in pearl millet [32], sorghum and rice [24]. The cost economics done in the present study indicated that conventional systems of chilli seed production registered the highest productivity (217 kg ha^{-1}) with gross return of Rs. 2,82,100 and net return of Rs. 2,03,850. Organic system of seed production with organic manures and biopesticides spraying (Neem seed kernel extract) recorded the gross return of Rs. 3, 12,000 and the net return of Rs. 2, 10,746. Benefit cost ratio, the index for economic viability and sustainability was higher in conventional system (1: 3.60) than in organic system (1: 3.08).

Conclusion

It was concluded that, for biopesticides sprayings, seed bioprimed with *P. fluorescens* with NSKE 5% to be best for organic plant protection measures to control pest, seed yield and quality in chilli

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