

Impact Analysis of IPM Programs in Basmati Rice by Estimation of Pesticide Residues

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Abstract Samples of Basmati rice grain, soil and water were collected, from IPM and non-IPM field trials conducted at four regions of Haryana, Uttar Pradesh and Uttarakhand in India, for pesticide residue analysis. Out of 45 soil samples collected, only four non-IPM samples indicated the presence of chlorpyrifos and endosulfan in the range of ND (<0.001) to 0.05 mg/kg. Carbendazim used at two locations of Dehradun and Kaithal was found below detectable limit (<0.05 mg/kg) in both IPM and non-IPM trials. Out of total 22 samples of water analyzed, chlorpyrifos was detected in samples from Kaithal and Pant Nagar in the range 0.003–0.006 $\mu\text{L/L}$, alpha –endosulfan isomer was detected in the range 0.005–0.03 $\mu\text{L/L}$ and the beta-isomer in the range 0.005–0.02 $\mu\text{L/L}$ in sample from Pant Nagar and Kaithal. The residues in all the grain sample of paddy were below detectable limit (<0.001 –0.05 mg/kg). The insecticides applied in IPM as well as non-IPM trials were found to be below maximum residue level (MRL).

Keywords Pesticides · Residues · Basmati rice · IPM · Non-IPM

Synthetic pesticides are an integral component of IPM programs. The rice crop is the second highest pesticide consuming crop in India. Basmati rice demands a higher price in domestic and export markets for its scented

characteristics, due to which farmers use repeated sprays of pesticides for managing the pests and maximizing yields.

Rice is attacked mainly with stem borer and leaf folder insect pests; blast and blight diseases besides few weeds like *Echinochloa* (Garg et al. 2004). An IPM protocol for Basmati rice was synthesized and validated in farmer's fields, in the states of Uttar Pradesh, Haryana and Uttarakhand, which are the major Basmati rice growing areas in India (Garg et al. 2007). Chemical crop protection inputs have been integrated along with cultural and biological techniques for managing insect pests and diseases of paddy. The IPM packages are location specific, and the IPM module involves seed treatment with carbendazim @ 2 g/kg seed along with spray of streptocycline (6 g/acre) or tricyclazole (1 g/L) or hexaconazole (1 L/ha) as spot application as per the symptoms of the disease. Need based application of carbendazim 50 WP is also recommended @ 2 g/L, as spray in IPM package. The pesticides are sprayed before flag leaf formation (50 days after transplantation). Methyl parathion, malathion, chlorpyrifos, hexaconazole, pretilachlor and endosulfan are widely used on paddy cultivation in the regions of Haryana and Uttarakhand in farmers field (non-IPM) as per package of practice of the region. It therefore becomes imperative to assess the pesticide residue levels in IPM as well as non-IPM managed crops Table 1.

This paper presents the reports of the assessment of the pesticide residues in the validation experiment of IPM module developed by Garg et al. (2007) for Basmati rice, so as to ensure the success of the module.

Materials and Methods

The samples of soil, water and rice grains at harvest were collected from IPM and non-IPM fields of Basmati rice

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Table 1 Retention times for various pesticides

S. no.	Pesticide	Retention time (min)
1	Methyl parathion	3.80
2	Malathion	4.56
3	Chlorpyrifos	4.83
4	Hexaconazole	6.77
5	Pretilachlor	7.17
6	Endosulfan α	6.47
7	Endosulfan β	7.67
8	Endosulfan sulfate	8.79

from the villages of Haryana, Uttrakhand and Uttar Pradesh and were analyzed for the presence of pesticides applied.

Forty five samples of soil, twenty two samples of water (common source for IPM and non-IPM fields) and thirty eight samples of rice grain, each from IPM and non-IPM trials were collected in triplicate including control from Tilwari village in Dehradun, Kaithal region in Haryana, Modipuram (UP) and Pant Nagar (Uttrakhand).

The samples of soil and irrigation water from Kaithal region were analyzed for the presence of chlorpyrifos, endosulfan, carbendazim, hexaconazole and pretilachlor as per the history of previous applications, while the samples of soil, water and rice grain from Dehradun region were analyzed for carbendazim. The samples of soil, water and rice grains from Modipuram and Pant Nagar region were analyzed for endosulfan, malathion, methyl parathion and carbendazim.

Untreated control samples (with no chemical treatment) of rice grain (50 g), soil (50 g) and water (500 mL) were spiked with a mixture of methyl parathion, malathion, chlorpyrifos, endosulfan, carbendazim, hexaconazole and pretilachlor at two fortification levels i.e. 0.1 and 1.0 ppm, for recovery studies.

50 g soil sample was sieved through 2 mm sieve and mixed thoroughly, into 250 mL Erlenmeyer flask, and 35 mL 0.2 M NH_4Cl solution (10.7 g/L) was added to it and allowed to stand for 15 min (AOAC Official Method 970.52). 100 mL hexane–acetone (1:1) was added and stoppered tightly and shaken overnight (≥ 12 h) on a reciprocal shaker at 180 rpm. The extract (avoiding aqueous-clay phase) was passed through 2–3 cm of activated Florisil packed on a glass column (30 cm $\times$ 15 mm id), pre-wet with petroleum ether 50 mL. The Erlenmeyer flask was rinsed with two 25 mL portions of hexane–acetone and decanted through column. The column was finally rinsed with 10 mL hexane–acetone. The combined eluate was collected in 1 L separatory funnel. 200 mL distilled water was added to separatory funnel and shaken gently for 30 s. Aqueous phase was drained into second separator and

extracted with 50 mL hexane. The hexane layers in first separator was combined and washed with 100 mL distilled water, drained and discard aqueous phase. Hexane extract was passed through 2 cm of anhydrous Na_2SO_4 , and concentrated to 10 mL volume in a rotary evaporator and analyzed the using GLC-ECD.

Rice grain sample (50 g) was weighed into homogenizer jar 200 mL acetonitrile–water (2 + 1) was added and homogenized for 5 min (AOAC Official Method 998.01). The extract was filtered under suction through Buchner funnel. The homogenizer was rinsed with two 25 mL portions acetonitrile–water (2 + 1), and the rinses were used to wash residues in Buchner funnel. The filtrate was transferred to 500 mL separatory funnel. The suction flask was washed with two 10 mL portions of acetonitrile–water (2 + 1), and the washes added to separatory funnel. 60 mL hexane was added to separatory funnel containing the extract. The funnel was shaken vigorously for 5 min with frequent venting. 200 mL of 4% aqueous NaCl (w/v) was added and mixed vigorously for 30 s. The layers separated and aqueous layer discarded. Hexane layer was passed through glass funnel containing cotton plug and ~ 15 g Na_2SO_4 , extract collected in 250 mL round-bottom flask. Separatory funnel rinsed with two 20 mL portions of hexane and rinses passed through same glass funnel into round-bottom flask. Contents of round-bottom flask evaporated to dryness on rotary evaporator at 40°C. Residue was redissolved in 10 mL hexane and transferred to 125 mL separatory funnel. The round-bottom flask was rinsed with two 5 mL portions hexane and added to separatory funnel. Acetonitrile (saturated with hexane) 30 mL was added and shaken vigorously with frequent venting for 5 min. The layers were allowed separate and acetonitrile phase drained into 250 mL round-bottom flask. The extraction repeated two more times. Acetonitrile extract evaporated to dryness on rotary evaporator at 60°C. Residue redissolved in 5 mL hexane and subjected to column clean up. A glass column packed with Florisil and prewashed with hexane (50 mL). The extract was transferred to column and allowed to fall until just above Florisil packing. The flask was rinsed with two 10 mL portions of hexane and added on to column and let run through column. The residues eluted with 150 mL of 50% eluting solvent (6% diethyl ether: petroleum ether), eluate collected at 3 mL/min in 250 mL round bottom flask. Eluate evaporated to less than 5 mL on rotary evaporator at 40°C and transferred to 20 mL stoppered test tube and analyzed by GLC.

The pH of water samples was found to be 8.2, adjusted to pH 7.0 by adding sulfuric acid (1N) (AOAC Official Method 990.06). Sample was transferred to separatory funnel, and 100 g sodium chloride added sealed and shaken to dissolve salt. 60 mL dichloromethane was added to the

beaker, swirled to rinse walls and then transferred to separatory funnel. The solution was shaken for 2 min with periodic venting. Organic layer was allowed separate from water phase for ≥ 10 min. Dichloromethane extract was collected in 500 mL Erlenmeyer flask containing about 5 g anhydrous sodium sulfate. A second 60 ml portion of dichloromethane was added to separatory funnel and extraction procedure repeated for the second time, and combined the extract in Erlenmeyer flask. The third extraction was performed in same manner. The flask was swirled to dry extract. Dichloromethane layer was decanted into concentrator flask. The remaining sodium sulfate was rinsed with two 25 ml portions of dichloromethane and rinses decanted into concentrator. The extract concentrated to 2 ml at 40°C. 10 mL MTBE was added and again concentrated to 2 mL. 5–10 mL MTBE was added one more time and concentrated to 2 mL. Finally the volume was made up with MTBE for analyzed by GLC-ECD.

The samples were analyzed by gas liquid chromatograph on Varian GLC CP-3800 fitted with electron capture detector. The column used was CPSil-5 CB (25 m $\times$ 0.25 mm $\times$ 0.25 μ) and the GLC parameters were, column: -170°C (3 min) @ $5^{\circ}\text{C}/\text{min}$, 220°C (5 min) then 270°C (10 min). The injector and detector temperatures were 250°C and 300°C , respectively. Carrier gas flow was maintained at 2 mL/min and make up gas 27 mL/min. Retention times and detection limit and of different pesticides are tabulated in Tables 2, 3, and Fig. 1.

Analysis of carbendazim in soil and water was carried out by HPLC. The above extracts were evaporated completely to remove the traces of hexane and made up in HPLC grade acetonitrile (5 mL). Carbendazim was analyzed on HPLC instrument Hitachi-La Chrom L-7100) with UV–VIS detector (L-7200) with autosampler (L-7400) column RP-18 (30 cm $\times$ 5 μ), was set at λ_{max} 250 nm, mobile phase was acetonitrile–water (50:50) @ 1.0 mL/min flow rate. The retention time was 3.32 min for carbendazim.

Table 2 Limit of detection of different pesticides in rice crop

S. no	Pesticide	Limit of detection (mg/kg)		
		Rice grain	Water ($\mu\text{L}/\text{L}$, ppb)	Soil
1	Methyl parathion	0.05	10	0.01
2	Malathion	0.05	10	0.01
3	Chlorpyrifos	0.01	1	0.001
4	Hexaconazole	0.05	1	0.01
5	Pretilachlor	0.05	1	0.01
6	Carbendazim	0.05	10	0.03
7	Endosulfan α	0.005	1	0.001
8	Endosulfan β	0.005	1	0.001
9	Endosulfan sulfate	0.05	5	0.05

Tentatively residue peaks were identified by comparing retention times from extract to those of standards solution. According to concentration of pesticide in the extracts, select the standard solution with peak area similar to those of the extracts. Inject a standard solution with peak area $\pm 25\%$ of analyte peak height. The sample was diluted and reanalyzed if analyte concentration exceeded 500 $\mu\text{g}/\text{mL}$.

Amounts of each pesticide in test extract was calculated of with the following equation:

$$\text{Residue, mg/kg} = C_{\text{Std}} \times (A_{\text{Ex}}/A_{\text{Std}}) \times (V_{\text{Std}}/V_{\text{Ex}}) \times (D/W)$$

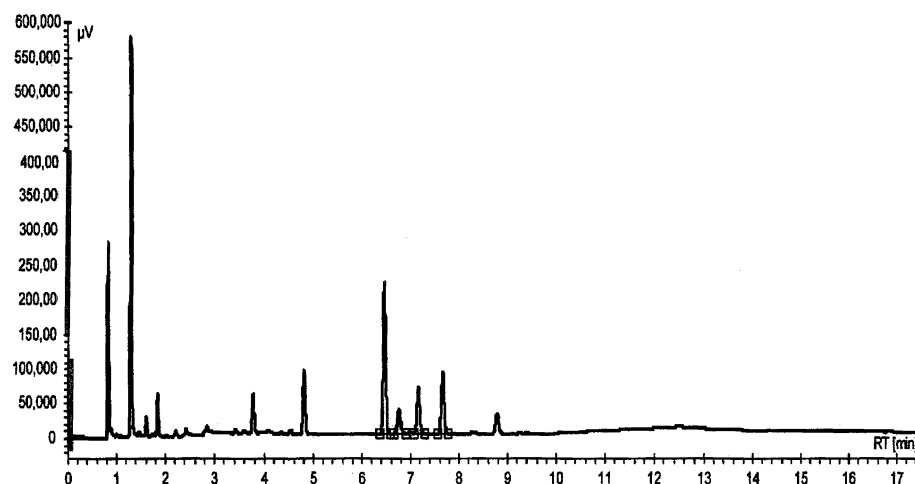
where C_{Std} is the standard concentration ($\mu\text{g}/\text{mL}$), A_{Ex} is the peak area in extract, A_{Std} is the peak area in standard, V_{Std} is the standard volume injected (μL), D is the dilution volume (mL), W is the test portion weight (g).

Results and Discussion

The recoveries of different pesticides, (Fig. 1) from paddy grain, straw, soil and water is presented in Table 4, and range from 70%–91%. The confirmation of pesticides used in the study were confirmed by GC Mass spectrum. The retention time (min) and m/z value for methyl parathion 4.36 min, m/z 57; malathion 6.71 min, m/z 127; chlorpyrifos 9.24 min, m/z 55; hexaconazole 11.03 min, m/z 149; petrialachlor 13.83 min m/z ; α -endosulfan 14.93 min, m/z 207; β -endosulfan 16.03 min, m/z 207; and endosulfan sulfate 21.59 min, m/z 207. In Kaithal region of Haryana, rice variety ‘Taraori Basmati’, is grown organically without any application of chemical pesticides in IPM trials while farmers were spraying pesticides viz., Cartap hydrochloride, chlorpyrifos, endosulfan, carbendazim, hexaconazole, anilophos and pretialachlor in non-IPM trials. Carbendazim was being applied as seed treatment as one of chemical component of IPM programs.). A similar multiresidue method was developed for rice grains by Gupta and Gajbhiye (2004). The field trials were conducted at Dehradun (variety Dehradun Basmati) during the year 2006–2007 for both IPM and non-IPM. In Modipuram region of Uttar Pradesh. Carbendazim was applied in IPM trials while endosulfan, malathion and methyl parathion was sprayed in non-IPM farmer’s fields. Similarly streptocycline was applied in IPM and endosulfan, malathion and methyl parathion was sprayed in non-IPM farmer’s fields in Pant Nagar at Uttarakhand. The residues of the pesticides in soil and water samples and rice grains of IPM trials (organic farming) were below the detectable limit in Kaithal area (Tables 5, 6, 7), while that of non-IPM trials showed traces of chlorpyrifos in 5 out of 16 samples in the range 0.003–0.006 $\mu\text{L}/\text{L}$ pesticides in water. The results

Table 3 Recovery of pesticides from different commodities

S.no.	Pesticide	Fortification level (ppm)	Percent recovery of pesticides			
			Paddy grain	Paddy straw	Paddy soil	Paddy water
1.	Malathion	1.0	80–82	83	84	85
2.		0.1	70–72	80	74	81
3.	Methyl parathion	1.0	77–82	77	84	81
4.		0.1	72–79	70	74	79
5.	Chlorpyrifos	1.0	8–91	88	82	88
6.		0.1	77–81	75	80	81
7.	Hexaconazole	1.0	79–85	84	82	85
8.		0.1	76–84	77	78	82
9.	Petrialachlor	1.0	77–81	78	76	82
10.		0.1	74–78	72	77	78
11.	Endosulfan (α,β , sulfate)	1.0	76–83	74	78	88
12.		0.1	74–79	76	75	84
13.	Carbendazim	1.0	72–81	78	75	82
14.		0.1	77–85	72	70	73

Fig. 1 GLC profile of pesticide used in Basmati rice**Table 4** Residues ($\mu\text{L/L}$) of pesticides in water from rice fields, canals

Location	No. of samples analyzed	Pesticides						
		Methyl parathion	Malathion	Chlorpyrifos	Hexaconazole	Pretilachlor	Endosulfan ($\alpha+\beta$ +end sulfate)	
Kaithal	16	–	–	0.003–0.006 (5 sample)	–	–	α -0.005–0.006 (2 samples) β -0.005–0.007 (2 samples)	
Dehradun	2	–	–	–	–	–	–	
Modipuram	2	–	–	–	–	–	–	
Pant Nagar	2	–	–	0.005 (1 sample)	–	–	α -0.03 β -0.02 (1 sample)	

are in concurrence with the first year data reported by Arora et al. 2008. A study conducted indicated the presence of some pesticides in ground water (Mukherjee and Gopal 2001).

Out of 45 soil samples collected, only four non-IPM samples indicated the presence of chlorpyrifos and endosulfan in the range of ND (<0.001) to 0.05 mg/kg, (Table 5). Carbendazim used at two locations of Dehradun

Table 5 Residues (mg/kg) of pesticides in soil under rice field

Location	No. of samples	Pesticides					
		Methyl parathion	Malathion	Chlorpyrifos	Hexaconazole	Pretilachlor	Endosulfan ($\alpha + \beta + \text{end sulfate}$)
Pant nagar –IPM	5	ND	ND	ND	ND	ND	ND
Pant nagar Non-IPM	5	ND	ND	0.001–0.05	ND	ND	ND
DDN-IPM	5	ND	ND	ND	ND	ND	ND
DDN-Non-IPM	5	ND	ND	0.003–0.005	ND	ND	0.003–0.02
Nagina-IPM	5	ND	ND	ND	ND	ND	ND
Nagina-Non-IPM	5	ND	ND	0.03–0.05	ND	ND	0.06–0.02
Kaithal-IPM	5	ND	ND	ND	ND	ND	ND
Kaithal-Non-IPM	5	ND	ND	0.002	ND	ND	0.07–0.01
Modipuram-IPM	5	ND	ND	ND	ND	ND	ND

Table 6 Residues (mg/kg) of pesticides in rice grains

Sample	No. of samples	Pesticides					
		Methyl parathion	Malathion	Chlorpyrifos	Hexaconazole	Pretilachlor	Endosulfan ($\alpha + \beta + \text{end sulfate}$)
Pant nagar–rice Grains-IPM	5	ND	ND	ND	ND	ND	ND
Pant nagar–rice Grains-non-IPM	5	ND	ND	ND	ND	ND	ND
Organic Pant Nagar	5	ND	ND	ND	ND	ND	ND
DDN-paddy-IPM	5	ND	ND	ND	ND	ND	ND
DDN-paddy-Non-IPM	5	ND	ND	ND	ND	ND	ND
Nagina-IPM	5	ND	ND	ND	ND	ND	ND
Nagina-Non-IPM	5	ND	ND	ND	ND	ND	ND
Modipuram-IPM	3	ND	ND	ND	ND	ND	ND

* ND methyl parathion <0.05 mg/kg; malathion <0.03 mg/kg; Chlorpyrifos <0.01 mg/kg; Hexaconazole <0.05 mg/kg; Petrialachlor <0.05 mg/kg

Table 7 Average residues of pesticides in rice grains

Sample	Pesticides (mg/kg)					
	Methyl parathion	Malathion	Chlorpyrifos	Hexaconazole	Pretilachlor	Endosulfan ($\alpha + \beta + \text{end sulfate}$)
Pant nagar –rice Grains-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
Pant nagar –rice Grains-non-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
Organic Pant Nagar	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
DDN-paddy-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
DDN-paddy-Non-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
Nagina-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
Nagina-Non-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05
Modipuram-IPM	<0.05	<0.03	<0.01	<0.05	<0.05	<0.001–0.05

and Kaithal was found below detectable limit (<0.05 mg/kg) in both IPM and non-IPM trials.

Out of total 22 samples of water analyzed, chlorpyrifos was detected in five samples from Kaithal region and one from Pant Nagar in the range 0.003–0.006 $\mu\text{L/L}$, endosulfan isomer was detected in the range 0.005–0.007 $\mu\text{L/L}$,

and the α -isomer in the range 0.002–0.007 $\mu\text{L/L}$ in one sample from Pant Nagar and in two samples from fields of Kaithal region (Table 6).

In Tilwari village of Dehradun region, farmers were not spraying any pesticides in rice crop; instead carbendazim was being applied as chemical component in IPM trials to

increase the productivity of rice crop. The non-IPM samples of rice grain, soil and water did not contain the residues of carbendazim in Dehradun region (Table 5, 6, 7). In Kaithal region of Haryana, rice variety 'Taraori Basmati', is grown organically without any application of chemical pesticides in IPM trials while farmers were spraying pesticides viz Cartap hydrochloride, chlorpyrifos, endosulfan, carbendazim, hexaconazole, and pretialachlor in non-IPM trials. In IPM trials of Modipraum region of Uttar Pradesh, carbendazim was applied while endosulfan, malathion and methyl parathion was sprayed in non-IPM farmer's fields. Similarly streptocycline was applied in IPM and endosulfan, malathion and methyl parathion was sprayed in non-IPM farmer's fields in Pant Nagar at Uttarakhand. Residues of pesticides applied at the early stages are below detectable limit in harvest time rice grains (Mukherjee and Gopal 2005).

The insecticides applied in IPM as well as non-IPM trials of both the regions were found to be below maximum residue level (MRL). The yield of Taraori Basmati rice in Kaithal region for IPM (organic farming) and non-IPM (farmers practice) field trials were 32.9 and 31.2 q/ha, respectively, while for Dehradun Basmati rice in IPM and non-IPM field trials, it was observed to be 21 and 18 q/ha, respectively. The impact of IPM and non-IPM trials on the basis of cost–benefit ratio is given in Table 8. Yield of IPM revealed a mean of 24.28 q/ha while in farmers field yield was 19.85 q/ha. Although the costs of inputs were higher it was realized in higher net returns and marginally higher cost benefit ratio of 1:3:37 as compared to 1:3:27 in farmers' field. National Centre for Integrated Pest Management is validating the IPM package in rice ecosystem in Basmati rice. The major Basmati growing states in India are Uttar Pradesh, Haryana and Uttarakhand. The major areas of rice are covered under the varieties Pusa Basmati-1 in Uttar Pradesh and Haryana. Taraori Basmati in Haryana and Dehraduni Basmati (T3) in Uttarakhand. Data on IPM modules on rice have also been reported by Kalode and Krishnaiah 1991 and Sharma et al. 2008. The IPM package synthesized by the centre was fine tuned for tackling the major problems of the area. The IPM module involved the following practices planting of dhaincha as a source of

green manure, seed treatment with *Trichoderma*, followed by planting of 2–3 seedlings/hill, application of fertilizers 60 N; 50 P; 40 K kg/ha and Zn SO₄ @ 25 kg/ha. Pheromone traps for YSB monitoring was set up. Further *Trichogramma japonicum* was released as need based. Spot application of carbendazim for control of blast and streptomycin for BLB. Weed management was done manually (Anonymous 2007). Systemic monitoring for insect pests, diseases and natural enemies was done periodically and application of pesticides was only need based. The farmers practice on the other hand had no green manuring and seed treatment, Planting of 5–8 seedlings/hill and fertilizer application was 20 N; 40 P; 0 K kg/ha. The farmers had no pheromone traps, no bio-agents application and also since no monitoring of pesticides. In the IPM module the incidence of stem borer recorded was 1.0%–2.5% as compared to 3.0%–8.5% in farmer's field. Similar findings were observed in case of leaf folder, IPM fields recorded 2.0%–5.0% infestation as compared to non-IPM practices in farmers field where infestation was high 5.0%–12.0%. Blast disease recorded in IPM and farmers field were 3.0%–5.0% and 3.0%–15.0%, respectively. Observations on natural enemies in IPM and non-IPM indicated spider fauna as dominant predator while lady bird beetle, *Xanthopimpia* sp and *Telenomus* sp.

The IPM module proposed for basmati rice in different regions, is evaluated for pesticide residues for the second year. The residues in all the grain sample of paddy were below detectable limit (<0.001–0.05 mg/kg, Table 7). The insecticides applied in IPM as well as non-IPM trials were found to be below maximum residue level (MRL). The main aim of the present investigations was to compare the pesticide load in IPM and non-IPM crops of rice fields. The four soil samples indicated presence of pesticides chlorpyrifos and endosulfan only in traces out of 45 soil samples collected from IPM and non-IPM fields of Basmati rice. Out of 22 samples of water analyzed, single sample from Pant Nagar and three from fields of Kaithal region were found to contain chlorpyrifos and endosulfan in traces, while the residues in all the grain sample of paddy were below detectable limit. Thus the insecticides applied in IPM as well as non-IPM trials were found to be below maximum residue level (MRL). The first year result also showed that harvest produce from IPM trials were safe to consume as the residues of insecticides were either below their MRL or not detected (Arora et al. 2008). Therefore the IPM package for Basmati rice can be safely adopted by the farmers.

Table 8 Cost benefit ratio of dehraduni Basmati rice production in IPM and non-IPM

S. no.	Variables	IPM	Non-IPM
1	Total cost including all inputs (Rs)	10,810	9,100
2	Mean yield (q/ha)	24.28	19.85
	Total returns (Rs)	3,36,420	29,775
	Net returns (Rs)	25,610	20,675
4	Cost Benefit Ratio	1:3:37	1:3:27

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