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The use of promising entomopathogenic fungi *M. anisopliae* for ecofriendly management of *Helicoverpa armigera* (Hub.) in chickpea

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Abstract

The effectiveness of entomopathogenic fungi *M. anisopliae* with the concentrations of 2×10^3 , 2×10^4 , 2×10^5 , 2×10^6 , 2×10^7 , 2×10^8 , 2×10^9 and 2×10^{10} conidia ml⁻¹ with 0.5 percent Jaggary were evaluated against *Helicoverpa armigera* (Hub) infestation on chickpea under field condition during 2020-21 and 2021-2022. The larval reduction percent was gradually increased with conidial concentration. The most effective conidial concentration was 2×10^{10} @ 5ml/lit water with 59.07 mean larval reductions percent, pod damage 7.33 percent and yielded 16.81 q/ha grain with 5.26 Rs. incremental cost benefit ratio.

Keywords: *Helicoverpa armigera*, *Metarhizium anisopliae*, concentrations and conidia

Introduction

Chickpea, *Cicer arietinum* (L.) is an important *Rabi* season pulse crop grown and consumed worldwide, especially in the Afro-Asian countries. It is also one of the major pulse crops cultivated and consumed in India and which is also known as Bengal gram. In India, chickpea account for about 45% of the total pulse production. Similar to the case of other pulses, India is the major chickpea producing country and it contributes for more than 75% of total world's chickpea production (Maurya and Kumar 2018) [1]. Chickpea plays an integral part of the cropping system in the farmer's field all over the country because it fits well in the crop rotation and mixed cropping system. It is multiple purpose crops and has the ability to grow under the conditions of low fertility and under varying conditions of soil and climate (Fikre and Rubiales 2014) [2].

In India chickpea is grown for decades. Chickpea fits really well in crop rotation and mixed cropping due to which it has become an integral part of our cropping systems all over the world because of its intrinsic value of higher protein content, nitrogen fixing ability and many diversified uses viz., green vegetables, germinated grain as breakfast, sweets and other relishing dishes and its indispensability as an alternate crop for crop diversification. Chickpea seed contains 18.22 percent protein, 16 percent total carbohydrates, 47 percent starch, 5 percent fat, 6 percent crud fiber, 6 percent-soluble sugar and 3 percent ash. (Jukanti *et al.*, 2012) [3]. Chickpea is native to India, Afghanistan and Ethiopia. It is out of the most important pulse crop in the world, cultivated in an area of 13.884 million hectares with a production of 13.652 million tones. In India, chickpea is grown in an area of 9.85 million hectares with production of 11.99 million tones. In India, Rajasthan is the largest chickpea growing state with an area of 2.46 million hectares with production of 2.66 million tones, followed by Maharashtra and Madhya Pradesh. Uttar Pradesh is the 4th largest producer with an area of 0.62 million hectares with a production of 0.85 million tones. (Anonymous, 2021) [4].

There are many pests infesting chickpea throughout the world in India it's about 57 species causing economic damage (Lal OP, 1996) [5]. Among them, gram pod borer, *Helicoverpa armigera* (Hub.) and cut worm, *Agrotis ipsilon* (Hufnager) are recognized as the major pests (Ranga and Shanower 1999) [6]. *Helicoverpa armigera* (Hubner) is among the most harmful agricultural pests. It is geographically widespread (Europ, Asia, Africa and Oceania) and is a highly polyphagous moth whose host species include various economically important crops such as cotton, corn chickpea, tomato, sorghum, sunflower (Akbulut *et al.*, 2003) [7].

Females lay their eggs on the fruits and flowers of these crops after hatching the larvae start to feed, causing significant agricultural damage. *Helicoverpa armigera* has been attributed as one of the serious pest status not only because of its ability to attack various hosts from various families but also for its resistance to insecticides (Cunningham *et al.*, 1998) [8].

Control of *H. armigera* is heavily dependent on the use of chemical pesticides. However, resistance to some commercially available insecticides has been detected in *H. armigera*. The increasing emergence of resistance problems means there is an urgent need for developing management strategies, which are less dependent on chemical insecticides and less conducive to the development of resistance problem. Therefore, use of microbial insecticides based on *Metarhizium anisopliae* (Green muscardine) plays an important role in the successful management of this pest.

Materials and Methods

The field evaluation of different concentrations of *M. anisopliae* to control *H. armigera* infestation on gram, *Cicer arietinum* (L.) was conducted in randomized block design with three replications during the Rabi season, 2020-21 & 2021-22 at Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut (UP). The crop was grown as sole crop during first fortnight of October and was raised by following all normal agronomical practices except plant protection measures.

Preparation of concentration

Sixty five gram of Sabouraud Dextrose Agar was used to prepare the growth substrate, which was dissolved in 1000 ml of distilled water and heated to boiling point. It was distributed in conical flasks and autoclaved in the oven for 20 min at 15 bar pressure, at 121 °C. In order to prepare the suspensions for the needs of the experiments, the fungi were cultured on 9 cm SDA petri dishes, secured with parafilm for protection against contamination and allowed to grow in the incubator for 15 days at 25 °C. Conidia were collected from the cultures after 15 days. The suspensions were prepared by scraping conidia from the surface of the petri dishes using a sterile metal hook. The conidia were transferred to 250 ml bottles containing 100 ml of sterile distilled water and 0.05% Tween 80. The conidial suspension was filtered through several layers of sterile muslin cloth before it was homogenized for 5 min using a magnetic stirrer. Finally, a Neubauer haemocytometer was used in an optical microscope (400x) to determine the desired doses i.e. 2×10^3 , 2×10^4 , 2×10^5 , 2×10^6 , 2×10^7 , 2×10^8 , 2×10^9 & 2×10^{10} (Lagogiannis *et al.*, 2020) [9]. These conidial formulations with 0.5 percent jaggary was sprayed with the knapsack sprayer at ETL (one or

two larvae per plant or two eggs per plant) of *Helicoverpa armigera* in chickpea.

Observations

A. Assessment of larval population

Ten plants per plot was randomly selected and tagged for recording observations. The pre count and post count were recorded a day before treatment and 3, 7, & 14 days after the application of the treatment. Efficacy of fungi at different concentration was calculated on the basis of larval reduction percent per ten plant after the treatment. The data on the larval reduction percent was subjected to suitable transformation and then statistical analysis.

$$\text{Larval reduction percent} = \frac{X_1 - X_2}{X_1} \times 100$$

Where,

X_1 = Larval population in untreated plot

X_2 = Larval population in treated plot

B. Assessment of pod damage

Pod damage was taken at the time of harvesting, total number of pods and number of damage pods taken and percent pod damaged was worked out by using following formula

$$\text{Percent pod damage} = \frac{\text{Number of affected pods/plant}}{\text{Total number of pods/plant}} \times 100$$

Incremental cost benefit ratio

Yield

The yield obtained in individual treatment of chickpea crop was recorded separately for assessing the efficacy of different concentrations. Data of yield kg/plot was converted into q ha⁻¹ with following formula.

$$\text{Grain yield (q/ha)} = \frac{\text{Grain yield (Kg/plot)} \times 100000 \text{ (m}^2\text{)}}{\text{Plot size (m}^2\text{)} \times 100}$$

Increase in yield over control was worked out by deduction the yield recorded in control plot from the yield of the respective treated plot. The monetary value of increase yield was computed in rupees using minimum support price of chickpea. A comparison of cost involved in different treatments was also calculated on the basis of maximum retail price printed on the pack taking account of the smallest pack size as reference. Net return for each treatment was calculated by deducting the cost of treatment from the monetary value of increased yield.

Incremental cost benefit ratio, net return per rupees invested, were calculated by using the following formula

$$\text{Incremental cost benefit ratio} = \frac{\text{Net return (Rs/ha)}}{\text{Cost of treatment (Rs/ha)}}$$

The incremental cost benefit ratio for all the treatments was worked out by considering the prevailing price of inputs like concentration of fungi, labour charge, rent of sprayer and market rate of gram etc.

Results and Discussion

All the treatments were found effective and significantly superior over control when the data of both years were pooled

(Table 1 and Fig 1). At one day before spray the mean larval population of *H. armigera* showed non-significant difference with the range of 9.33 to 10.16 per ten plants.

After third day after first and second spray the highest pooled reduction percent of *H. armigera* was found 38.78 and 51.08 in the treatment 2×10^{10} @ 5ml/lit water. After first and second spray the next effective treatments were 2×10^9 @ 5ml/lit water with 34.07 & 47.48, 2×10^8 @ 5 ml/lit water with 27.63 & 44.99, 2×10^7 @ 5 ml/lit water with 24.38 & 42.58, 2×10^6 @ 5 ml/lit water with 19.49 & 38.95, 2×10^5 @ 5 ml/lit water with 14.69 & 36.49 and 2×10^4 @ 5 ml/lit water with 8.10 & 31.61 percent. The treatments 2×10^3 @ 5 ml/lit water showed minimum reduction percent after and second spray with 1.70 & 27.99 percent larval reduction.

After seven days of first spray the highest pooled larval reduction of *H. armigera* was 51.94 percent in the treatment of 2×10^{10} @ 5 ml/lit water and the sequence of effectiveness were 2×10^9 @ 5 ml/lit, 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit and 2×10^4 @ 5 ml/lit water with 46.01, 41.54, 38.52, 32.68, 29.67 and 26.74 percent respectively. The lowest larval reduction was observed in the treatment 2×10^3 @ 5 ml/lit water with 23.82 percent. The same pattern found after fourteenth days of spray with highest pooled larval reduction 75.97 and the lowest reduction 22.85 percent in the treatments 2×10^{10} @ 5 ml/lit and 2×10^3 @ 5 ml/lit water respectively.

After seven days of second spray the pooled larval reduction percent ranged from 33.95 to 58.73 per ten plants. The highest larval reduction observed in the treatment 2×10^{10} @ 5 ml/lit water with 5 per 8.73 cent followed by 2×10^9 @ 5 ml/lit, 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit and 2×10^4 @ 5 ml/lit water with 54.03, 50.45, 46.84, 42.22, 39.89 and 36.31 percent respectively. The minimum reduction noticed in treatment 2×10^3 @ 5 ml/lit water 33.95 percent.

Almost same pattern was observed after fourteen days of first and second spray, the highest pooled larval reduction percent of *H. armigera* was 75.97 and 77.85 percent recorded in the plots treated with 2×10^{10} @ 5 ml/lit water and the next effective treatment was 2×10^9 @ 5 ml/lit water with 70.92 and 72.35 percent followed by 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit and 2×10^4 @ 5 ml/lit water with 68.37, 64.59, 56.93, 49.40, 44.28 and 66.89, 57.80, 55.56, 52.17, 46.54 percent respectively. The least pooled larval reduction was recorded 41.74 and 41.13 percent in the plot treated with 2×10^3 @ 5 ml/lit water after first and second spray.

The overall mean of pooled larval reduction percent ranged from 17.00 to 65.49 percent. The highest reduction percent was recorded 65.49 percent in the treatment of 2×10^{10} @ 5ml/lit water followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit and 2×10^4 @ 5 ml/lit water with 54.14, 49.98, 45.79, 40.97, 37.05 and 32.27 percent respectively. The lowest reduction percent was found in the treatment of 2×10^3 @ 5 ml/lit water with 28.55 percent.

The statistically analyzed pooled data revealed that the percent pod damage caused by this pest ranged from 7.33 to 37.17 percent. The treatment 2×10^{10} @ 5 ml/lit water found best among all the treatments with minimum pod damage 7.33 percent followed by 2×10^9 @ 5 ml/lit, 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit, 2×10^4 @ 5 ml/lit and 2×10^3 @ 5ml/lit water with 8.67, 10.83, 12.67, 15.50, 18.33, 20.67 and 22.67 percent respectively. The highest pod damage was noticed in control with 37.17 percent.

The present findings also get support from the observations of Savita *et al.* (2015) ^[10] who reported that the mortality of *H. armigera* population gradually increase with conidial concentration of the fungus and mentioned 1.10 larvae/five plants with pod damage 9.50 percent and 14.50 q/ha yield at 1×10^{10} conidial concentration. Similar observations made by Agale *et al.* (2017) ^[11] who reported that higher conidial concentration of *M. anisopliae* 4.3×10^3 conidia/ml cause 50 percent larval mortality. The present findings also agreement with the finding of Lagogiannis *et al.* (2020) ^[9] who reported that the high yield concentrations 10 and 10 conidia/ml of all three fungi pathogenic with larval mortality ranging between 87 to 100 percent at nine days and they also mentioned the lower doses of 10^3 , 10^4 and 10^5 which had produced zero mortality by day three, only the higher doses 10^6 , 10^7 and 10^8 induced the mortality significantly different from the control. The larval mortality at 13 days was 100 percent @ 10^8 conidial concentrations. This finding also accordance with Phukon *et al.* (2014) ^[12] who reported reduction in fruit damage up to 87.01 percent over control at 1×10^9 conidial concentration of *M. anisopliae* and 3.8 larvae/15 plants after 7 DAT at vegetative stage and 1.2 larvae/15 plants at fruiting stage.

Effect of different treatments on grain yield in chickpea during Rabi, 2020-2021 & 2021-2022

The all the concentration of entomopathogenic fungus *M. anisopliae* gave higher yield when the data of both years were pooled and found superior over control. The maximum pooled grain yield of 16.18 q/ha was recorded with treatment 2×10^{10} @ 5 ml/lit water and 2×10^9 @ 5 ml/lit water was second best treatment with 15.56 q/ha grain yield. The next treatments in order were 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit, 2×10^4 @ 5 ml/lit and 2×10^3 @ 5 ml/lit with grain yield 14.49, 13.61, 12.79, 12.10, 11.31 and 11.05 q/ha respectively.

The present result corroborates with Savita *et al.* (2015) ^[10] who reported that the among the all concentration of *M. anisopliae* the treatment 1×10^{10} cfu per ml recorded 9.50 percent pod damage and produced 14.50 q/ha grain yield. The finding also accordance with Spoorthi *et al.* (2017) ^[13] who treated the plot with 2×10^9 cfu/ml @ 2g/lit water and found 14.58 q/ha grain yield. The present finding also in confirmation with Tekam *et al.* (2018) ^[14] treated plot with 1×10^9 cfu/ml and found 12.26 percent pod damage and this finding also supported.

Table 1: Efficacy of *M. anisopliea* in field condition against *H. armigera* Rabi 2020-21, 2021-22 (Pooled)

Treatments	Dose ml/lit water	Larval reduction percent/10 plants							Overall Mean	
		No. of larva/10 Plants	First Spray				Second Spray			
		1 DBT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT		
$2 \times 10^3 + 0.5\%$ jaggery	5	9.99 (18.42)	1.70 (7.48)	23.82 (29.18)	41.74 (40.22)	27.99 (31.93)	33.95 (35.61)	42.13 (40.45)	28.55 (32.29)	
$2 \times 10^4 + 0.5\%$ jaggery	5	9.66 (18.13)	8.10 (16.52)	26.74 (31.09)	44.28 (41.70)	31.62 (4.20)	36.31 (37.03)	46.54 (42.97)	32.27 (34.59)	
$2 \times 10^5 + 0.5\%$ jaggery	5	9.66 (18.13)	14.69 (22.50)	29.67 (33.00)	49.40 (44.63)	36.49 (37.15)	39.89 (39.15)	52.17 (46.22)	37.05 (37.48)	
$2 \times 10^6 + 0.5\%$ jaggery	5	10.16 (18.61)	19.49 (26.19)	32.68 (34.86)	56.93 (48.94)	38.95 (38.59)	42.22 (40.51)	55.56 (48.17)	40.97 (39.77)	
$2 \times 10^7 + 0.5\%$ jaggery	5	9.66 (18.13)	24.38 (29.56)	38.52 (38.35)	64.59 (53.47)	42.58 (40.72)	46.84 (43.17)	57.80 (49.46)	45.79 (42.57)	
$2 \times 10^8 + 0.5\%$ jaggery	5	10.16 (18.61)	27.63 (31.69)	41.54 (40.10)	68.37 (55.76)	44.99 (42.09)	50.45 (45.24)	66.89 (54.86)	49.98 (44.98)	
$2 \times 10^9 + 0.5\%$ jaggery	5	9.66 (18.13)	34.07 (35.69)	46.01 (42.68)	70.92 (57.36)	47.48 (43.54)	54.03 (47.29)	72.35 (58.27)	54.14 (47.35)	
$2 \times 10^{10} + 0.5\%$ jaggery	5	10.16 (18.61)	38.87 (38.55)	51.94 (46.09)	75.97 (60.65)	51.08 (45.59)	58.73 (50.01)	77.85 (61.90)	59.07 (50.20)	
Control	-	9.33 (17.78)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	
CD @ 5%		NA	0.767	1.106	1.818	1.182	1.340	1.818	1.29	
S.Em±		NA	0.254	0.336	0.601	0.391	0.443	0.601	0.42	

Angular transformation

DBT – Day before Treatment, DAT – Day After Treatment.

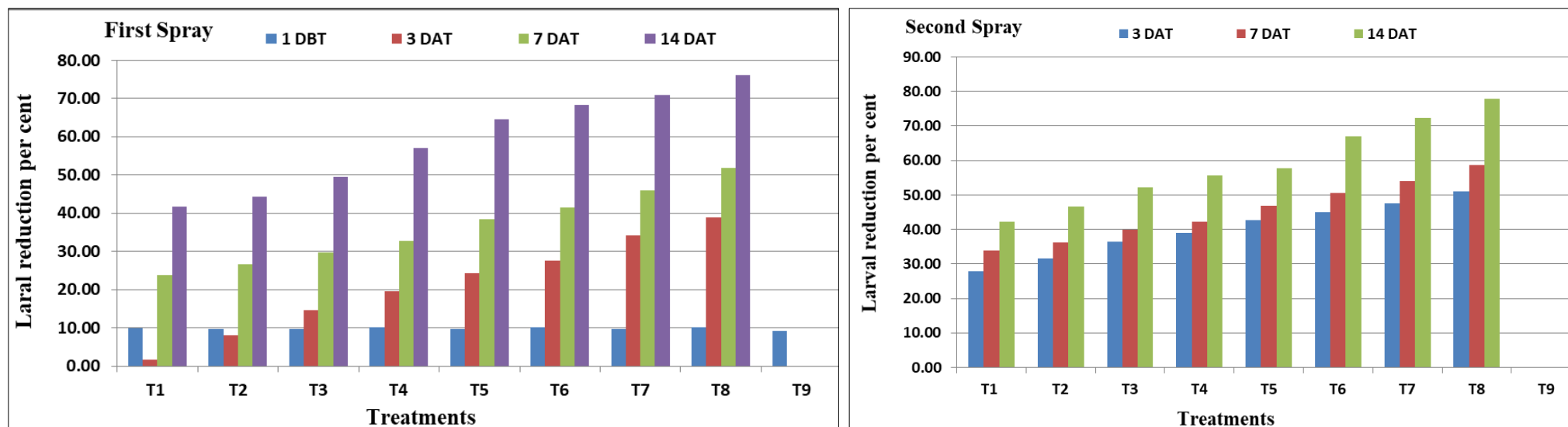


Fig 1: Effect of different treatments on population of *H. armigera* after first and second spray during Rabi, 2020-2021 & 2021-2022 (Pooled).

Effect of different treatments on incremental cost benefit ratio

Working out the cost benefit ratio of both years' pooled data, revealed that the maximum incremental cost benefit ratio was recorded in the treatment of 2×10^{10} @ 5 ml/lit water with 5.26 and followed by 2×10^9 @ 5 ml/lit, 2×10^8 @ 5 ml/lit, 2×10^7 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^6 @ 5 ml/lit, 2×10^5 @ 5 ml/lit and 2×10^4 @ 5 ml/lit with incremental cost benefit ratio of 4.43, 3.67, 3.01, 2.30, 1.65 and 1:0.70,

respectively. The minimum ICBR of 0.45 was found in the treatment of 2×10^3 @ 5 ml/lit water.

The present findings are agreement with Spoorthi *et al.* (2017)^[13] who treated the plot with 2×10^9 cfu/ml @ 2 g/lit water and found increased yield over control was 5.23 q/ha and incremental cost benefit ratio 1: 9.01. The present finding also agreed with Savita *et al.* (2015)^[10] observed that application of different concentration of *M. anisopliae* increase the yield and incremental cost benefit ratio.

Table 2: Yield, economics and incremental cost benefit ratio of different treatments in chickpea during Rabi, 2020-21, 2021-22 and pooled

Tr. No.	Name of treatments	Dose/lit water	Yield (q/ha)	Increase yield over control (q/ha)	Value of increase yield (Rs./ha)	Cost of treatments (Rs./ha)	Net profit (Rs./ha)	ICBR
T ₁	$2 \times 10^3 + 0.5\%$ jaggery	5	11.05	0.82	4089.75	2830.00	1259.75	0.45
T ₂	$2 \times 10^4 + 0.5\%$ jaggery	5	11.31	1.08	5386.50	3175.00	2211.50	0.70
T ₃	$2 \times 10^5 + 0.5\%$ jaggery	5	12.10	1.87	9326.63	3520.00	5806.63	1.65
T ₄	$2 \times 10^6 + 0.5\%$ jaggery	5	12.79	2.56	12768.00	3865.00	8903.00	2.30
T ₅	$2 \times 10^7 + 0.5\%$ jaggery	5	13.61	3.38	16857.75	4205.00	12652.75	3.01
T ₆	$2 \times 10^8 + 0.5\%$ jaggery	5	14.49	4.26	21246.75	4550.00	16696.75	3.67
T ₇	$2 \times 10^9 + 0.5\%$ jaggery	5	15.56	5.33	26583.38	4895.00	21688.38	4.43
T ₈	$2 \times 10^{10} + 0.5\%$ jaggery	5	16.81	6.58	32817.75	5240.00	27577.75	5.26
T ₉	Control	-	10.23	-	-	-	-	-

Labour charge Rs. 350/Labour, Rent of sprayer Rs. 100/sprayer, Cost of produce Rs. 4987.50

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