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Assessing Pigeonpea Genotypes for Resistance against *Maruca vitrata* Fabricius and its Sustainable Management through Biorationals

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ABSTRACT

Background: The spotted pod borer (SPB), *Maruca vitrata* Fabricius is a key insect pest of pigeonpea, *Cajanus cajan* in India and semi-arid tropics. The SPB has developed insecticide resistance to most of the chemical insecticide and its habitat is expanding. Identifying a SPB resistant genotype will help in sustainable management of SPB and in attaining nutritional security.

Methods: Forty seven pigeonpea genotypes were screened during *kharif* 2015 to 2017 at ICAR-IIPR, Kanpur based on % pod damage and Pest Resistance Susceptibility Rating (PRSR). The 4 biorationals were tested against SPB in ICPL 87. Further its molecular identity was confirmed by phylogenetic analysis of partial *Cox 1* gene.

Result: Two indeterminate genotypes (Pusa2001 and Pusa33) were identified as SPB resistant based on lowest mean PRSR (1.7). The PRSR 2.0 was scored by AL-15 and LRG-30. Spinosad 45SC has recorded significantly lowest mean pod damage (6.67%) and highest mean yield (4.36 kg/35 m^2) in the field efficacy trial. The phylogenetic analysis of *Cox 1* gene from Kanpur population (KY559101) with 19 other *M. vitrata* populations in India revealed that it is more similar to Raichur population (KT070892) and it confirms *M. vitrata* has same ancestral homology in India.

Key words: *Cox 1*, Flower webber, Legume pod borer, Phylogenetic analysis, Pod damage, Resistance screening.

INTRODUCTION

Pigeonpea, *Cajanus cajan* (L.) Millspaugh is 2nd important grain legume in tropics and sub-tropics after chickpea, *Cicer arietinum* L. In India it is cultivated in an area of 4.80 Million-hectare, with a production and productivity of 4.28 million tonnes and 892Kg/ha during 2020-2021 (Anonymous 2021). Pigeonpea seeds are rich in protein and many insect pests infest it. Among the biotic constraints, insect pests such as legume pod borer, *Maruca vitrata* Fabricius, gram pod borer, *Helicoverpa armigera* Hubner, pod fly, pod bugs, blue butterfly, blister beetle and plume moth inflict a heavy yield loss as they infest the pigeonpea flowers or pods or seeds. The avoidable yield loss due to *M. vitrata* infestation in pigeonpea genotype is 84.68% in determinate genotype (MN1) and it is 39.55% in indeterminate type (PAU 881) (Mahalle and Taggar, 2017). The legume pod borer is a key insect pest, infesting > 73 leguminous crops such as *Cajanus cajan*, *Vigna unguiculata*, *V. radiata*, *V. mungo*, *phaseolus*, *Lablab purpureus* L., *etc* (Srinivasan *et al*. 2021). The application of following insecticides like profenophos 50 EC, DDVP 76 EC, methomyl 40 SP and chlorpyriphos 20 EC helps in managing this insect pest. Keeping in view of environmental protection the CIBRC has banned DDVP from 31st December 2020. Further application of insecticide is very tough in pigeonpea as it is taller at the time of flowering and podding. Hence an alternative strategy like host plant resistance (HPR) is a viable and sustainable option for managing this polyphagous insect pest. The host plant resistance against this insect pest has been worked out in some of the legume crops in India such as cowpea (Jakhar *et al*., 2017), *L. purpureus* (Mallikarjuna *et al*., 2009; Sujitra and Srinivasan 2012), *V. mungo* (Umbarkar *et al*., 2011; Cheema *et al*., 2017) and pigeonpea (Sunitha *et al*., 2008a; Saxena *et al*., 1996). Unlike other crops pigeonpea is having different growth habits (determinate and indeterminate) and maturity groups (short duration, medium duration and long duration). Therefore while screening for legume pod borer resistance in pigeonpea these factors has to be considered and accordingly a screening programme as to be formulated. There exists a lack of targeted resistance breeding programme against *M. vitrata* in India and abroad (Saxena *et al*., 2016). The persistence effort to critically evaluate the resistance in existing genotypes against this insect pest in pigeonpea is the pioneer step for a targeted resistance

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breeding. The identification of a resistant genotype will help the resistance breeding programme to increase the productivity of pigeonpea crop in India. Hence, the present investigation was carried out to identify the resistance level among the commonly available pigeonpea genotypes. It is necessary for any resistance breeding programme to have the pest identity lucidly; so the *M. vitrata* population prevailed during the screening programme was characterized using *Cytocrome C oxidase* gene (*Cox1*). Apart from screening for insect resistance a field experiment for identifying a best biorational for *M. vitrata* management of this hidden pest was also carried out in this study.

Materials and Methods

Screening of pigeonpea genotypes

Forty-seven pigeonpea genotypes were sown at new research campus of ICAR-IIPR, Kanpur (26°51′N, 80°24′E) for screening it against spotted pod borer, *Maruca vitrata* Fabricius. The screening was done for 3 consecutive years during *kharif* 2015, 2016 and 2017 by using the pest resistance susceptibility rating (PRSR) given by Lateef and Reed (1983). The seeds were sown on 23rd to 24th standard meteorological week every year in 2 m row for each genotype with spacing of 0.75 m × 0.30 m. The pigeonpea genotype, ICPL 87-susceptible check for *M. vitrata* was sown after every two pigeonpea entries in field to ensure uniform *M. vitrata* population infestation to all genotypes. Pre-emergence herbicidal spray of pendimethalin was done within 24 hrs of sowing. The recommended agronomic practices like weeding, irrigation and earthing up were done as and when required. The experimental field was maintained insecticide free throughout the cropping period to facilitate natural infestation of *M. vitrata*. The per cent pod damage was estimated by counting total number of healthy and damaged pods from 5 randomly tagged pigeonpea plants of each genotype. The *M. vitrata* damaged pods were identified by presence of bore holes with remnant silken webs with faecal material on the pod wall. The mean of five plants were used for calculating pest resistance/susceptibility percentage (PRSP) as follows:

$$\text{PRSP} = \frac{\text{PD\% in susceptible check-PD\% in test line}}{\text{PD\% in susceptible check}} \times 100$$

The PRSR rating for a genotype were given as per estimated PRSP as shown below:

PRSR	PRSP
1	100%
2	75 to 99.99%
3	50 to 75%
4	25 to 50%
5	10 to 25%
6	-10 to 10%
7	-25 to -10%
8	-50 to -25%
9	≤-50%

Biorational management

The pigeonpea genotype, ICPL 87 was sown during *kharif* 2017 in RBD design at ICAR-IIPR New Research Campus with individual plot size of 5 m × 7 m for evaluating 4 biorationals (Pongamia oil 1%, Emamectin benzoate 5 SG @ 0.3 ml/L, Spinosad 45SC @ 0.16ml/L and Indoxacarb15.8 EC @ 0.5 ml/L) in comparison with an untreated control. Each treatment was replicated thrice. At the time of harvest, 50 pods from each replication was collected and the per cent pod damage due to *M. vitrata* was counted and its average per cent pod damage was estimated.

$$\text{Pod damage \% (PD\%)} = \frac{\text{Number of damaged pods}}{\text{Total number of pods}} \times 100$$

Per cent reduction in pod damage over control (PROC) =

$$\frac{\text{PD\% in control-PD\% in test entry}}{\text{PD\% in control}} \times 100$$

The PD% data was subjected to ANOVA using OPSTAT software (Sheoran *et al*., 1998).

Phylogenetic analysis of *M. vitrata*

The *M. vitrata* larvae from the field were collected during 40th Standard meteorological week (Sujayanand *et al*. 2020) and it was reared until pupation on pigeonpea flowers and pods. The *M. vitrata* DNA was isolated from the virgin adult by dissecting its thorax and legs. The protocol given by Hipura®insect DNA purification kit was followed for DNA isolation and purification. The *Cox 1* primers LEPF and LEPR as reported by Hebert *et al*. (2003) were used for amplifying the ~690 bp of mitochondrial cytochrome oxidase I (*Cox 1*) region of insect DNA. Further the PCR amplified product was sequenced by sanger dideoxy method (Chromous Biotech Pvt. Ltd Bangalore). The results were trimmed using BLAST tool and it was submitted to NCBI database. The ~690 bp nucleotide sequence was subjected to phylogenetic analysis of *M. vitrata Cox 1* sequences reported from other regions of the world by using MEGA 7.0 software. The representative nucleotide sequences of *M. vitrata* from different locations of India (Table 1) were downloaded from NCBI in FASTA format (Chatterjee *et al*. 2019). Phylogenetic tree for *Cox1* was constructed in MEGA 7.0 using maximum likelihood method considering1000 bootstrap replications under distance models Tamura3-parameter (T92) (Tamura, 1992).

Results and Discussion

Screening of pigeonpea genotypes

The legume pod borer, *M. vitrata* is emerging as serious insect pest in major grain legumes and it has evolved resistance against organophosphorous and synthetic pyrethroid insecticides in India (Sreelakshmi *et al*., 2015). Hence with this background the present experiment on identification of resistant genotypes initiated during *kharif* (2015). The per cent pod damage during *kharif* (2015), (2016) and (2017) varied from 12.26 to 89.81; 0 to 35 and 12.90 to 64.29 respectively (Table 2). The PRSR for 47

Table 1: Field screening of pigeonpea genotypes against spotted pod borer, *M. vitrata* during *Kharif* 2015, 2016, 2017.

Genotype	2015			2016			2017			Mean	Growth
	PD %	PRSP%	PRSR	PD%	PRSP%	PRSR	PD%	PRSP%	PRSR	PRSR	habit#
AL-15	22.33	75.14	2	6.67	80.95	2	15.38	76.07	2	2.0	DT
AL-201	30.42	66.13	3	0.00	100.00	1	17.54	72.71	3	2.3	IDT
TJT 501	42.88	52.25	3	13.33	61.90	3	23.19	63.93	3	3.0	IDT
BANAS	36.13	59.77	3	0.00	100.00	1	22.41	65.14	3	2.3	IDT
CORG 9701	45.49	49.34	4	0.00	100.00	1	17.65	72.55	3	2.7	IDT
PUSA 84	35.98	59.94	3	0.00	100.00	1	24.39	62.06	3	2.3	IDT
PUSA 2001	12.26	86.35	2	0.00	100.00	1	14.06	78.13	2	1.7	IDT
PUSA 992	41.35	53.96	3	10.32	70.52	3	23.73	63.09	3	3.0	IDT
PUSA 33	18.41	79.50	2	0.00	100.00	1	12.90	79.93	2	1.7	IDT
PUSA 991	28.82	67.91	3	4.76	86.39	2	15.69	75.60	2	2.3	IDT
TAT-10	49.73	44.62	4	0.00	100.00	1	40.68	36.73	4	3.0	IDT
JA-4	13.93	84.49	2	11.57	66.94	3	13.56	78.91	2	2.3	IDT
MANAK	14.89	83.42	2	0.00	100.00	1	50.00	22.23	5	2.7	IDT
UPAS 120	27.85	68.99	3	4.17	88.10	2	16.28	74.68	3	2.7	SDT
GT-100	47.79	46.79	4	6.67	80.95	2	28.57	55.56	3	3.0	DT
PARAS	26.27	70.75	3	0.00	100.00	1	18.33	71.48	3	2.3	IDT
ICPL 151	57.75	35.70	4	0.00	100.00	1	46.67	27.41	4	3.0	DT
VAMBAN 1	28.68	68.06	3	0.00	100.00	1	33.33	48.15	4	2.7	DT
ICPL 87	89.81	0.00	6	35.00	0.00	6	64.29	0.01	6	6.0	DT
BDN 708	47.31	47.32	4	0.00	100.00	1	30.00	53.34	3	2.7	IDT
LRG 38	48.67	45.81	4	8.33	76.19	2	15.00	76.67	2	2.7	IDT
ICPL 85063	30.96	65.53	3	11.43	67.35	3	20.00	68.89	3	3.0	IDT
BRG 708	47.70	46.88	4	0.00	100.00	1	25.00	61.11	3	2.7	IDT
ASHA	43.49	51.57	3	0.00	100.00	1	25.00	61.11	3	2.3	IDT
LRG 30	39.22	56.33	3	0.00	100.00	1	13.33	79.26	2	2.0	IDT
BDN-2	25.28	71.85	3	15.08	56.92	3	33.33	48.15	4	3.3	IDT
CO-5	32.44	63.88	3	0.00	100.00	1	20.00	68.89	3	2.3	IDT
VIPULA	40.34	55.08	3	5.56	84.13	2	36.67	42.97	4	3.0	IDT
VAMBAN 2	48.26	46.26	4	0.00	100.00	1	16.67	74.08	3	2.7	IDT
ICPL 332	31.68	64.73	3	0.00	100.00	1	30.00	53.34	3	2.3	IDT
CO-6	50.13	44.18	4	0.00	100.00	1	15.00	76.67	2	2.3	IDT
BSMR 736	47.14	47.51	4	0.00	100.00	1	33.33	48.15	4	3.0	IDT
BSMR 853	37.92	57.78	3	0.00	100.00	1	16.67	74.08	3	2.3	IDT
LRG 41	49.75	44.61	4	6.06	82.68	2	13.33	79.26	2	2.7	IDT
MARUTHI	36.72	59.11	3	8.33	76.19	2	20.00	68.89	3	2.7	IDT
IPAC 8	33.47	62.73	3	0.00	100.00	1	33.33	48.15	4	2.7	IDT
ICPL 84023	15.72	82.49	2	6.67	80.95	2	20.00	68.89	3	2.3	DT
ICPL 7124	48.00	46.55	4	0.00	100.00	1	33.33	48.15	4	3.0	DT
DSLR 129	37.44	58.31	3	7.41	78.84	2	25.00	61.11	3	2.7	SDT
ICPL 91045	53.38	40.56	4	0.00	100.00	1	33.33	48.15	4	3.0	IDT
ICPL 88039	51.43	42.74	4	0.00	100.00	1	31.67	50.74	3	2.7	IDT
MN-5	78.11	13.03	5	15.08	56.92	3	41.67	35.19	4	4.0	DT
ICPL 87154	45.91	48.88	4	0.00	100.00	1	21.67	66.30	3	2.7	DT
WD-5	23.15	74.23	3	4.76	86.39	2	18.33	71.48	3	2.7	IDT
ICPL 11255	49.13	45.29	4	0.00	100.00	1	25.00	61.11	3	2.7	DT
ICPL 88034	51.96	42.15	4	0.00	100.00	1	41.67	35.19	4	3.0	IDT
ICPL 20340	53.56	40.36	4	0.00	100.00	1	35.00	45.56	4	3.0	DT

DT- Determinate; IDT- Indeterminate; SDT- Semi-determinate.

genotypes ranged from 2 to 6; 1 to 6 and 2 to 6 respectively for *kharif* (2015), (2016) and (2017). *kharif* 2016 had recorded lower *M. vitrata* infestation than *kharif* (2015) and (2017). The mean PRSR ranged from 1.7 to 6.0.

The rigorous field screening of 47 pigeonpea genotypes belonging to different growth habitats (*i.e.* 10 indeterminate genotypes 3 semi determinate genotypes and 34 indeterminate genotypes) against *M. vitrata* infestation during 3 consecutive seasons *viz*., *kharif* 2015 to 2017 has resulted in identification of 2 Indeterminate genotypes (Pusa 2001 and Pusa 33) and a determinate genotype (AL-15) having lowest pest resistance susceptibility rating (PRSR) and identified as *M. vitrata* resistant. The present finding agrees with the findings of Kumar *et al*. (2015) wherein they had reported Pusa 33 to have highest protease inhibitor (PPI) activity and the purified PPI had recorded 46% larval mortality and also extended larval period of *H. armigera* by 12 days.

Two genotypes *viz*., AL-15 and LRG-30 had recorded second lowest PRSR (2.0). The present study is the first of its kind to have identified 2 determinate genotypes in pigeonpea for tolerance to *M. vitrata viz*., AL-15 and ICPL84023. The remaining least susceptible genotypes (12 genotypes) were of indeterminate type. The present result supports the findings of Sharma *et al*. (1999) and Gangwar and Bajpai (2007) wherein they had reported lowest larval and pupal mass and *M. vitrata* infestation (0.9%) in ICPL 84023.

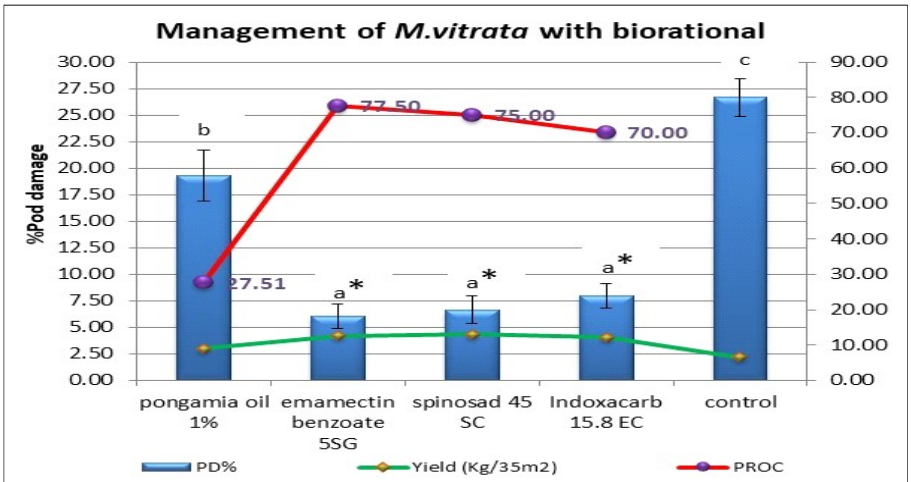
Twelve genotypes (ICPL 84023, JA 4, AL 201, Banas, Pusa 84, Pusa 991, Paras, Asha, C0-5, Co-6, ICPL 332 and BSMR853) had recorded third lowest PRSR (2.3). The present result supports the findings of Rathod *et al*. (2014) were they had recorded lowest mean *M. vitrata* larva per plant (1.8/plant) and pod damage (18.59%) in BSMR 853. Further the present result is supporting the findings of Anatharaju and Muthiah (2008) and Saxena *et al*. (2016) who had reported that ICPL 332 (13 larva/6 plants) and C0-6 (18.33 larva/6 plants) had lowest *Maruca* infestation and *Helicoverpa armigera* tolerance. The genotype ICPL332 WR has resistance to fusarium wilt also (Sharma, 2016). Ambidi *et al*. (2021) reported that ICPL 332 WR and BSMR 853 as least preferred genotypes by pod borer. Further, ICPL 332 WR has recorded lowest percent pod damage (19.1) and highest tannin content (12.20 mg/g) and established their inverse correlation as reported by Jat *et al*. (2018). The identified 16 resistant genotypes can be deployed in resistance breeding programme for developing interspecific crosses with *C. scarabaeoides*. Some of wild derivatives from *C. scarabaeoides* were found to exhibit tolerance against *H. armigera* under no choice assay conditions (Sujayanand *et al*., 2019). Thus the present finding will assist in developing pod borer resistance genotypes conferring resistance to both *H. armigera* and *M. vitrata*.

Biorational management

The entomopathogenic fungus (*Beauvaria bassiana* and *Metarizhium anisoplae*) or bacteria (*Bacillus thuringiensis*) were tried for the management of *M. vitrata* (Srinivasan *et al*., 2014; Sujayanand *et al*. 2018); however, the locally available strains are least effective (Sunitha *et al*. 2008b). Hence in the present study efforts were made to study the efficacy of botanical (Pongamia oil) / biorationals (Spinosad 45 SC, Emamectin benzoate 5SG and Indoxacarb 15.8 EC). The mean percent pod damage due to *M. vitrata* infestation in ICPL 87 among the 4 biorational treatments and untreated control varied from 6.00 to 26.67% (Fig 1) during *kharif* 2017. The treatments T_2 to T_4 (Emamectin benzoate 5 SG, Spinosad 45SC and Indoxacarb 15.8 EC) had recorded

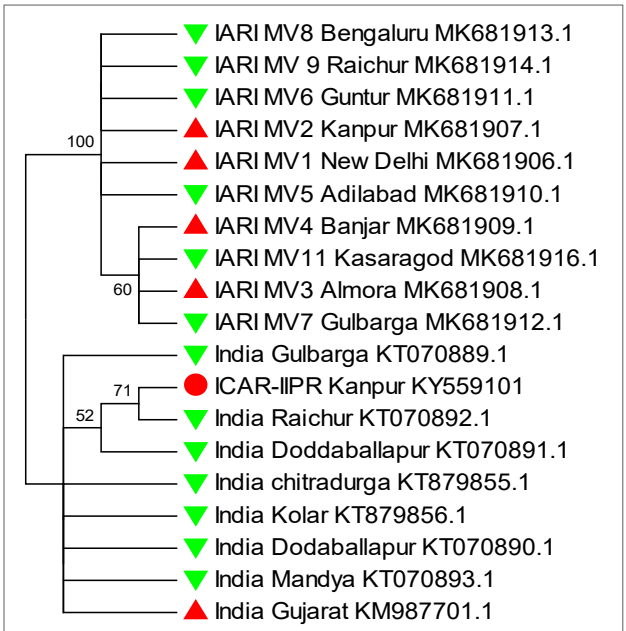
Table 2: Details of the *M. vitrata* populations used in phylogenetic analysis.

Accession number	Specific location	Latitude	Longitude	Host crop
MK681913.1	Bengaluru, India	77.5946 E″	12.9716 N	*Cajanus cajan*
MK681914.1	Raichur, India	77.3439 E	16.2120 N	*Cajanus cajan*
MK681911.1	Guntur, India	80.4365°E	16.3067°N	*Cajanus cajan*
MK681907.1	Kanpur, India	80.3319°E	26.4499°N	*Cajanus cajan*
MK681906.1	New Delhi, India	77.1025°E	28.7041°N	*Cajanus cajan*
MK681910.1	Adilabad, India	79.5603°E	19.0809°N	*Cajanus cajan*
MK681909.1	Banjar, India	77.3441°E	31.6377°N	*Cajanus cajan*
MK681916.1	Kasargod, India	74.9852°E	12.5102°N	*Cajanus cajan*
MK681908.1	Almora, India	79.2902°E	29.8150°N	*Cajanus cajan*
MK681912.1	Gulbarga, India	79.5941°E	17.9689°N	*Cajanus cajan*
KT070889.1	Gulbarga, India	76.7831°E	17.3919°N	Not available
KT070892.1	Raichur, India	77.3244°E	16.2051°N	*Cajanus cajan*
KT070891.1	Doddaballapur, India	77.6022°E	13.3112°N	*Phaseolus vulgaris*
KT879855.1	Chitradurga, India	76.3773°E	14.2358°N	Not available
KT879856.1	Kolar, India	78.1121°E	13.1422°N	Not available
KT070890.1	Dodaballapur, India	77.5293°E	13.1805°N	*Lablab purpureus* L.
KT070893.1	Mandya, India	76.5731°E	12.3933°N	*Lablab purpureus* L.
KM987701.1	Anand, India	72°95′E	22°55′N	*Vigna unguiculata*
KY559101.1	Kanpur, India	80.2490°E	26.5186°N	*Cajanus cajan*



*Shows significant statistical difference (p=0.0006) of treatments. All values are mean±SE. N=3 replicates per treatment. Error bars represent standard errors.

Fig 1: Management of spotted pod borer with biorationals in ICPL87.



Molecular phylogenetic analysis by maximum likelihood method
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3-parameter model [17]. The tree with the highest log likelihood (-1644.6886) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The analysis involved 19 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 611 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [39].

Fig 2: Phylogenetic analysis of *Maruca vitrata* fabricius.

significantly lower percent pod damage (6.00, 6.67 and 8.00% respectively) than T_5 (untreated control: 26.67%) and T_1 (Pongamia oil 1%: 19.33%). The per cent reduction over control varied from 27.51 to 77.5. The lowest was recorded from the treatment (T_1) Pongamia oil 1% (27.51) while the highest per cent reduction over control was recorded from (T_2) Emamectin benzoate 5 SG (77.50) and Spinosad 45SC (75.00). The present finding is in congruence with reports of Bharathi *et al*. (2019) and Pandey and Das (2016) wherein they had reported that pongamia oil and pongamia soap was least effective in *Lablab purpureus* var *typicus* and JA-4, respectively. The present result supports the findings of Sambathkumar *et al*. (2015) as they had reported 12.6 larval webbing per 10 plants in pongamia soap alone whereas 9.8 larval webbings per 10 plants in pongamia soap + indoxacarb treatment, 3.2 larval webbing per 10 plants in 2 sprays of Indoxacarb alone.

The pigeonpea mean seed yield per plot varied from 2.18 kg/35 m^2 to 4.36 kg/35 m^2. The lowest yield was recorded from untreated control (T_5: 2.18 kg/35 m^2) while the highest yield was recorded from Spinosad 45SC (T_3:4.36 kg/35 m^2). The present study confirms the superiority of Spinosad 45 SC, Emamectin benzoate 5SG and Indoxacarb 15.8 EC as very effective in reducing the percent pod damage as reported by Sunitha *et al*. (2008b) and Sreekanth and Seshamahalakshmi (2012).

Phylogenetic analysis of *M. vitrata*

The insect DNA was isolated, amplified and BLAST analysis of Cox *1*nucleotide sequence (690 bp) confirmed it as *Maruca vitrata*. The nucleotide sequences with accession number: KY559101was subjected to neighbor joining analysis; which revealed that the Kanpur population shared more similarity with Raichur population and Doddaballapur population (Fig 2). The results showed that there is no much

difference among the Indian population and they may be derived from same ancestral origin. The present finding confirms the findings of Chatterjee *et al*. (2019) and Periasamy *et al*. (2015). Further, Kim *et al*. (2016) reported that their Korean population shares homology with Asia-African cluster and different from the remaining 2 other populations from America and Oceania. Thus, the Indian population is not genetically different in different agroecological regions of pigeonpea crop.

CONCLUSION

The present investigation concludes that 2 pigeonpea genotypes *viz*., Pusa 2001 and Pusa 33 as spotted pod borer, *M. vitrata* resistant based on lowest mean PRSR 1.7 among the 47 pigeonpea genotypes screened. This was followed by 2 other genotypes (AL-15 and LRG-30) with a score of 2.0. Third lowest mean PRSR (2.3) was scored by 12 other genotypes (JA 4, AL 201, Banas, Pusa 84, Pusa 991, Paras, Asha, ICPL 84023, C0-5, Co-6, ICPL 332 and BSMR853). Spinosad 45SC is more effective in managing the pest efficiently. The phylogenetic analysis of partial *Cox1* gene from Kanpur population with other *M. vitrata* from pigeonpea revealed it as more similar to Raichur population and they share common ancestral homology. Thus, resistant pigeonpea genotype (Pusa 2001 or Pusa 33) accompanied by periodical monitoring and spraying of Spinosad 45 SC at ETL can help in sustainable management of *M. vitrata*. The present findings will help in developing IPM package for pigeonpea or in resistance breeding programme.

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Conflict of interest

The authors declare that the research was conducted in the absence of commercial or financial relationships that could be construed as a potential conflict of interest.

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