could be attributed to the substantially elevated catalase levels. Therefore, multiple short exposures to hydrogen peroxide in culture resulted in the significant up-regulation of antioxidant defence mechanism and a strong grip over oxidative stress, causing an increase in tyrosine phosphatase activity and consequent abrogation of the EGF response. Thus it is conceivable that repetitive oxidative stress attenuated the ability of the EGFR to respond to EGF due to the activation of tyrosine phosphatases, and inhibition of this early step in EGF signalling resulted in the blockage of the downstream components.

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Isolation of plant growth-promoting strains of *Bradyrhizobium* (*Arachis*) sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut

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Among ten strains of *Bradyrhizobium* (*Arachis*) sp. in peanut, only three produced siderophore and IAA, and exhibited phosphate solubilization *in vitro*. *Bradyrhizobium* strains AHR-2^{amp+}, AHR-5^{amp+} and AHR-6^{amp+} showed antagonistic activity against *Macrophomina phaseolina*. Peanut seeds coated with *Bradyrhizobium* strains were significant by enhanced seed germination, seedling biomass, nodule number, nodule fresh weight, average nodule weight compared to uninoculated and uninfected controls. These findings confirm the antagonistic as well as plant growth-promotory properties of *Bradyrhizobium* strains.

BIOLOGICAL control is an environment-friendly strategy to reduce crop damage caused by plant pathogens¹. Biological control of soil-borne pathogens with antagonistic bacteria and fungi has been intensively investigated². Rhizosphere-resident antagonistic microorganisms are ideal biocontrol agents, as the rhizosphere provides the front-line defence for roots against infection by the pathogens³. Biocontrol research has gained considerable attention and appears promising as a viable alternative to chemical control strategies.

The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legumes in terms of biological nitrogen fixation has been a main focus in the recent past. Obviously, rhizobia are known to increase nodulation and nodule weight in legumes along with increase in host plant growth and development⁴, besides protecting roots from the attack of

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pathogens due to production of diverse microbial metabolites like siderophore⁵, rhizobitoxin⁶, plant growth enhancement through IAA production, uptake of phosphorus and other minerals⁷. A few strains of rhizobia are reported to inhibit sclerotia germination of Sclerotium rolfsii⁸ and colony growth of Phytophthora megasperma⁹. Rhizobium meliloti and Bradyrhizobium japonicum bacterized seeds are known to have reduced Macrophomina phaseolina infection¹⁰.

M. phaseolina (Tassi) Goid. is a major pathogen of more than 500 hosts, including peanut¹¹. Use of Bradyrhizobia has dual advantage compared to that of fluorescent pseudomonads, as the former assimilate atmospheric nitrogen besides killing deleterious phytopathogens. Hence, this work was designed to assess the biocontrol potential of strains of Bradyrhizobium (Arachis) sp. against M. phaseolina causing charcoal rot of peanut.

Root-nodulating strains of *Bradyrhizobium* were isolated from *Arachis hypogaea* (peanut) by standard microbiological techniques¹². Bradyrhizobia were maintained on yeast extract mannitol agar (YEMA) at 4°C. Strains were characterized according to *Bergey's Manual of Determinative Bacteriology*¹³ and checked for their ability (infectivity) to establish nodule formation in peanut seedlings. *M. phaseolina* was isolated from diseased seeds of peanut by blotter technique¹⁴ and maintained on Czapek Dox agar at 4°C.

Antagonistic activity of bradyrhizobial strains was tested against *M. phaseolina* by using dual culture technique¹⁵. Five-day-old mycelial discs (5 mm dia.) of *M. phaseolina* were placed at four corners on the modified YEMA by including 2% sucrose. Exponentially grown *Bradyrhizobium* strains (48 h in YEM broth) were spotted in the centre of agar plates and incubated at 28 ± 1°C for five days. Inhibition in radial growth of test fungus was measured.

Fresh culture filtrate (10, 20 and 40 ml) of Bradyrhizo-bium was transferred separately in 250 ml conical flask containing 90, 80, 60 ml, 2% sucrose plus YEM broth respectively. Two mycelial discs (5 mm) from five-day-old culture of M. phaseolina were also transferred into each flask. The flasks were incubated at $28 \pm 1^{\circ}$ C for seven days. This experiment was also done with auto-claved culture filtrate of Bradyrhizobium strains for 20 min. Flasks containing only mycelial disc in the medium devoid of culture filtrate served as control. After seven days of incubation, fresh mycelial mat was harvested and dried at 85° C for 24 h to constant weight for obtaining fungal growth.

Siderophore production by *Bradyrhizobium* strains was tested by using chrome-azurol S (CAS) assay medium¹⁶. *Bradyrhizobium* strains were spread over YEMA and incubated at 28°C for 96 h. Thereafter, a thin layer of CAS reagent in 0.7% agar was spread over the colonies of *Bradyrhizobium* and the plates were re-incubated as earlier. Formation of yellow-orange halo around the

colonies indicates siderophore production¹⁷. The bacterial culture filtrate (48 h) was used to determine the presence of catechol and hydroxamate-type siderophores^{18,19}.

Production of hydrocyanic acid (HCN) was assayed by the modified method of Miller and Higgins 20 . Exponentially grown bacterial cultures were separately streaked on tryptic soya agar (TSM) and YEMA plates supplemented with 4.4 g l $^{-1}$ glycine with simultaneous supplementation of a filter paper soaked in 0.5% picric acid in $1\%\ Na_2CO_3$ in the upper lid of petri dishes. The plates were sealed with parafilm. Control plates did not receive inoculum. After incubation at $28\pm1^{\circ}C$ change in colour from yellow to light brown, moderate (brown) or strong (reddish-brown) indicated HCN production.

The log phase cultures (48 h) of bacterial strains were raised separately in 5 ml YEM broth, incubated for 24 h and centrifuged at 7000 rpm for 15 min at 4°C. The supernatant was collected and finally passed through $0.2 \, \mu \text{m}$ millipore filter. Two drops of o-phosphoric acid were added to 2 ml of supernatant to develop pink colour.

The plates containing Pikovskya's $agar^{21}$ were spot inoculated by bradyrhizobial strains and incubated at 28 ± 1 °C for five days. Formation of clear zone around the colonies indicated phosphate solubilization.

Antibiotic sensitivity test of *Bradyrhizobium* strains was performed using disc impregnated with antibiotics of different known concentrations (Hi-media, Mumbai, India). The discs were placed in four corners over the surface of seeded *Bradyrhizobium* strains in YEMA plates. The plates were incubated at $28 \pm 1^{\circ}\text{C}$ for 72 h. Ampicillin (5 µg ml⁻¹) produced maximum inhibition zone. Ampicillin-resistant marker strains were developed by subjecting the cultures successively from 1 to $10 \, \mu\text{g ml}^{-1}$. The bacterial strains (amp⁺) thus obtained were used for seed bacterization and root colonization experiment.

The method of Arora et al. 17 was adopted for seed bacterization. Peanut seeds were surface-sterilized with 0.5% NaOCl solution for 3-5 min, rinsed in sterilized distilled water and dried overnight under a sterile air stream. Cells of Bradyrhizobium strains were grown under continuous shaking condition (150 rpm) on YEM broth at 28 ± 1°C for 72 h. Each culture was separately centrifuged at 7000 rpm for 15 min at 4°C. The culture supernatant was discarded and the pellets were washed with sterile distilled water (SDW) and resuspended in SDW to obtain a population density of 108 cfu ml⁻¹. The cell suspension was mixed with 1% carboxymethylcellulose (CMC) solution. The slurry was coated separately on the surface of peanut seeds and allowed to air-dry overnight in aseptic condition. Care was taken to avoid clumping of seeds. The seeds coated with 1% CMC slurry without bacterial strains served as control.

Sterile earthen pots (24 cm \times 12 cm \times 12 cm) were filled with sterilized sandy loam soil (0.24% total organic matter, 0.097% total organic C, 37% water-holding capacity,

pH 6.4). Inoculum of M. phaseolina was prepared by multiplying it on moist and sterilized oat (Avena sativa) grains in a 500 ml flask²⁰. The oat grain-based culture of M. phaseolina was separately mixed in soil so as to make the inoculum level of approximately 107 cfu/g soil. Seeds bacterized with Bradyrhizobium (Arachis) strains AHR-2, AHR-5 and AHR-6 along with their respective nonbacterized seeds (control) were sown in eight sets of treatment: treatment I - soil inoculated with Bradyrhizobium strains AHR-2; treatment II - soil inoculated with Bradyrhizobium strains AHR-5; treatment III - soil inoculated with Bradyrhizobium strains AHR-6; treatment IV - soil inoculated with Bradyrhizobium strains AHR-2 + M. phaseolina; treatment V – soil inoculated with Bradyrhizobium strains AHR-5 + M. phaseolina; treatment VI - soil inoculated with Bradyrhizobium strains AHR-6 + M. phaseolina; treatment VII - soil inoculated with M. phaseolina; treatment VIII – uninoculated soil as control (with non-bacterized seeds and without M. phaseolina in non-sterile soil). Three seeds per pot were sown and after 15 days, thinning was done to raise only single healthy plant in each pot. The plants were irrigated with tap water whenever required. Seed germination (%) was noted on 15th day of sowing. Seedling biomass and nodule weight were recorded after 30 and 60 days of sowing. Disease incidence was recorded as percentage of the plants showing charcoal rot symptom after 60 days. Doses of Bradyrhizobium suspension (10⁸ cfu ml⁻¹) were periodically applied following soil drench method at 15 and 30 days after sowing.

Bradyrhizobium AHR-2^{amp+}, AHR-5^{amp+} and AHR-6^{amp+} were used to study root colonization. After 15 days plants were carefully uprooted with a shovel and soil particles adhering to the roots were collected on a sterile filter paper. One gram of rhizosphere soil was serially diluted in SDW to determine cfu/g soil¹². The serially diluted suspension was properly mixed with YEMA medium containing 10 μg ml⁻¹ ampicillin plus 25 μg ml⁻¹ cycloheximide to control fungal growth and incubated at 28 ± 1°C for 72 h. After five days of incubation at 30°C, colony-forming units of *M. phaseolina* were determined on modified YEMA medium by spread plate technique.

The data were analysed statistically by using analysis of variance (ANOVA), LSD and regression coefficient.

Ten strains of *Bradyrhizobium* (*Arachis*) sp. were isolated from fresh and healthy nodule of peanut. The morphological, biochemical and physiological characteristics, generation and nodulation on peanut (host plant) confirmed that the bacterial isolates belong to *Bradyrhizobium* cowpea miscellany group.

Bradyrhizobium strains AHR-2, AHR-5 and AHR-6 inhibited the growth of M. phaseolina in vitro on modified YEMA plates at 28 ± 1 °C. Bradyrhizobium AHR-2 caused maximum growth inhibition (72%) of M. phaseolina compared to AHR-5 and AHR-6 strains. Inhibition in colony growth of test fungus increased corresponding to

incubation time (Figure 1). Maximum growth inhibition was recorded after five days of incubation. Maximum value of regression coefficient in case of AHR-2, AHR-5 and AHR-6 was 0.899, 0.902, 0.909 respectively, which was significant at P > 0.01 level of ANOVA. Fungal growth inhibition of M. phaseolina by Rhizobium was observed in vitro $^{6.8,17,23}$.

Culture filtrate (48-h-old) of bradyrhizobial strains significantly (P > 0.01) inhibited the growth of M. phaseolina by 48–56% compared to the control. Fresh, free culture filtrate was more effective in inhibiting the fungal growth than autoclaved culture filtrate. When the quantity of culture filtrate was raised in broth, a marked decline in fungal growth was recorded. The fungal growth was completely inhibited at 40% level of culture filtrate (Table 1). Hence, the presence of a toxin in autoclaved culture filtrate of Bradyrhizobium cannot be ruled out. The inhibitory properties of rhizobial culture filtrate containing rhizobitoxin have been reported by Chakraborty and Purkayastha⁶. Rhizobitoxin is an important compound involved in symbiosis between rhizobia and legumes²⁴.

Bradyrhizobium strains AHR-2^{amp+}, AHR-5^{amp+} and AHR-6^{amp+} showed production of orange-yellow halo around the colonies (Table 2). Larger halo was formed around the colony of strain AHR-2 than those of AHR-5 and AHR-6. Development of pink colour with sodium nitrite indicated the production of catechol-type of siderophore by the strains¹⁸. A similar observation was recorded earlier¹⁷. Disease reduction involving siderophore-mediated competition is generally believed to be one of the antagonistic interactions that results in the exclusion of fun-

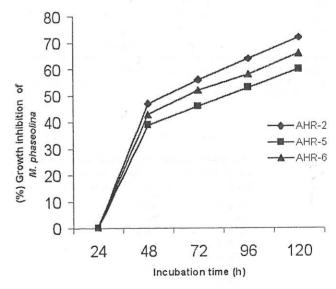


Figure 1. In vitro inhibition (%) of radial growth of M. phaseolina due to Bradyrhizobium strains AHR-2, AHR-5 and AHR-6. Values are mean of 5 replicates and significant at 1% level of ANOVA. Regression coefficients of AHR-2 = 0.899; AHR-5 = 0.902 and AHR-6 = 0.909.

gal pathogen in the rhizosphere due to reduction in the availability of iron for spore germination and hyphal growth²⁵.

No change in the colour of filter paper after 4–5 days of incubation at $28 \pm 1^{\circ}\text{C}$ showed the incapability of the strains to produce HCN in vitro (Table 2). On the other hand, a pink colour developed when o-phosphoric acid was added to culture supernatant of Bradyrhizobium strains. A dominant population of AHR-2, AHR-5 and AHR-6 strains was isolated from peanut, which produced IAA (Table 2). Prevost et al. 26 reported that 96% rhizobial strains synthesized IAA and enhanced plant growth.

Formation of clear zone around the colony in Pikovsky's agar medium showed phosphate solubilization by the bacteria. About 60% *Bradyrhizobium* strains were capable of phosphate solubilization. The strains AHR-2, AHR-5 and AHR-6 exhibited a higher rate of phosphate solubilization than the other strains (Table 2). High population of phosphate-solubilizing rhizobia in rhizosphere and increased plant growth have also been reported²¹.

Bradyrhizobium strains AHR-2, AHR-5 and AHR-6 increased seed germination by 25, 11 and 15% respectively (Table 3). Germination of bacterized seeds sown in

Table 1. Effect of culture filtrate of *Bradyrhizobium* strains against *M. phaseolina* after seven days of incubation

	Mycelial dry weight (mg per 50 ml)* Bradyrhizobium strain					
-						
Culture filtrate (%) of - Bradyrhizobium	AHR-2	AHR-5	AHR-6			
10	37 ± 0.1 (52 ± 0.3)	52 ± 0.2 (89 ± 0.4)	49 ± 0.2 (73 ± 0.4)			
20	17 ± 0.1 (33 ± 0.2)	23 ± 0.1 (64 ± 0.5)	21 ± 0.1 (59 ± 0.4)			
40	0	5 ± 0.1 (16 ± 0.1)	2 ± 0.1 (19 ± 0.1)			

Values are mean of three replicates; \pm Standard deviation; Values in parentheses are the effect of autoclaved culture filtrate; *, Significant at P > 0.01 level of ANOVA.

M. phaseolina-infested soil significantly increased when compared to control. A decline in germination of non-bacterized seeds by 13% was recorded in M. phaseolina-infested soil compared with control (Table 3). The data suggested that enhanced seed germination of bacterized seeds was due to production of antifungal metabolites by bradyrhizobial strains.

Population of *Bradyrhizobium* strains AHR-2, AHR-5 and AHR-6 increased nodule weight sevenfold higher than the control, and seeds coated with *Bradyrhizobium* strains AHR-2, AHR-5 and AHR-6 also enhanced nodule fresh weight more than sixfold when sown in *M. phaseolina*-infested soil (Table 2). A similar observation was recorded in other legumes^{17,27}.

Bradyrhizobium strains AHR-2, AHR-5 and AHR-6 increased seedling biomass by 83, 63 and 71% respectively, compared to the control. These values are significant at 1, 5 and 1% level of LSD respectively, compared to M. phaseolina (Table 3). Incidence of charcoal rot caused by M. phaseolina decreased in case of seeds bacterized with Bradyrhizobium strains AHR-2, AHR-5 and AHR-6, and enhanced early vegetative growth in peanut. This could be due to the resident microflora of seed surface as most of the seed-borne and soil-borne pathogens infect the plants during seed germination. The bradyrhizobial strains coated on seeds may reduce infection of seedlings during the germination process.

Bradyrhizobium AHR-6 increased nodule number in M. phaseolina-infested soil compared to the control. On the other hand, the number of nodules per plant decreased by 38% in M. phaseolina-infested soil. Seeds coated with Bradyrhizobium strains (AHR-2, AHR-5 and AHR-6) significantly (P > 0.01) increased nodule number per plant by 231, 169 and 163% respectively, and the strains did not decrease nodulation in M. phaseolina-infested soil in comparison with control (Table 3). The strains AHR-2, AHR-5 and AHR-6 significantly (P > 0.01) increased nodule weight by 231, 133 and 141% in non-infested soil and 154, 145, 128% in M. phaseolina-infested soil respectively (Table 3). Results indicated that bradyrhizobial

Table 2. Production of siderophore HCN, IAA and phosphate solubilization, antagonism against *M. phaseolina* by *Bradyrhizobium* strains AHR-2, AHR-5, AHR-6

Bradyrhizobium	Generation	Siderophore	HCN	IAA	Phosphate	Antagonism agains	
train time (h)		production	production	production production		M. phaseolina	
AHR-1	10.7	1 - 1	_	_	+	_	
AHR-2	10.9	+	_	+	++	+	
AHR-3	10.3	8. <u>-</u>	_		_	-	
AHR-4	10.5	_	-		=	_	
AHR-5	10.7	+	-	+	++	+	
AHR-6	10.6	+	-	+	++	+	
AHR-7	10.7	5 - -1	1 <u>-1</u>	+	-		
AHR-8	10.9		8-3	_	-	_	
AHR-9	10.6	_	_	_	+		
AHR-10	10.5	_	_	+	+	.	

^{+,} Positive reaction; -, Negative reaction; + +, 10 mm radial clearing zone of phosphate solubilization; +, 5 mm radial clearing zone of phosphate solubilization.

strains enhanced plant growth and nodule number in peanut seedlings. Similar observations were reported in other legumes^{21,27}.

Population of Bradyrhizobium strains (AHR-2, AHR-5 and AHR-6) increased in the first 30 days after seed sowing, but slightly declined thereafter. Bacterial population (cfu g⁻¹) in rhizosphere soil inoculated with Bradyrhizobium strains AHR-2+M. phaseolina decreased by 26% compared to Bradyrhizobium AHR-2 alone after 60 days. There was a slight variation in population of Bradyrhizobium strains (cfu g-1) in rhizosphere soil. The results suggested that Bradyrhizobium strains (AHR-2, AHR-5, AHR-6) were good root colonizers even in the presence of M. phaseolina (Table 4). The findings of root colonization by Bradyrhizobium strains AHR-2, AHR-5 and AHR-6 also support the ability of these strains for biological control and plant growth enhancement. Population of M. phaseolina declined in rhizosphere soil of peanut in the presence Bradyrhizobium strains AHR-2,

AHR-5 and AHR-6. The higher population of *Bradyrhizobium* strains in peanut rhizosphere indicated that the strains are potential root colonizers due to their presence in considerable number, and can multiply using the degradation products of the nodules as substrate. Available literature revealed that the bacterial symbiont enhances the host legume growth over other bacteria and showed synergism, if they are able to reduce root disease⁹. Therefore, it could be more judicious if legumes are inoculated with host-specific rhizobial species, which provide not only nitrogen but also some degree of protection against seed-borne and soil-borne phytopathogens.

The observation clearly suggested that the siderophoreand IAA-producing and phosphate-solubilizing *Bradyrhizobium* strains (AHR-2, AHR-5 and AHR-6) are good root colonizers and possess a strong antagonistic activity against *M. phaseolina*. *Bradyrhizobium* strains have been found effective antagonists *in vitro* and *in vivo*, besides enhancing seed germination, seedling biomass, nodu-

Table 3. Effect of bacterization with *Bradyrhizobium* strains AHR-2, AHR-5, AHR-6 on seed germination, seedling biomass, nodule fresh weight, nodules per plant and weight per nodule of peanut in *M. phaseolina*-infested soil

Treatment		Seedling biomass (g/plant)		Nodule fresh weight (mg/plant)		Nodule	
	Seed germination - (%)					No. plant ⁻¹	Wt plant-1
		30 days	60 days	30 days	60 days	(number)	(mg) ^a
M. phaseolina	60	3.12	7.91	12	21	10	0.21
Bradyrhizobium AHR-2	86**	8.21*	18.12**	252**	401**	53**	7.56**
Bradyrhizobium AHR-5	77*	7.72*	16.13*	147**	296**	43**	6.88**
Bradyrhizobium AHR-6	80**	9.91*	16.97**	147**	297**	42**	7.07**
Bradyrhizobium AHR-2 + M. phaseolina	84**	7.98*	16.12*	148**	298**	40**	7.45**
Bradyrhizobium AHR-5 + M. phaseolina	73*	7.52*	15.07*	143**	295**	41**	7.19**
Bradyrhizobium AHR-6 + M. phaseolina	75*	7.61*	15.12*	144**	295**	44**	6.70**
Control	69	5.72*	9.91 ^{ns}	25	47	16	2.93

Values are mean of five replicates; **, Significant at 0.01 level of LSD compared to M. phaseolina treatment; *Significant at 0.05 level of LSD compared to M. phaseolina treatment; *, Average value of nodule weight per plant.

Table 4. Population of *Bradyrhizobium* strains AHR-2, AHR-5, AHR-6 and *M. phaseolina* in presence of each other

	Log cfu g-1 in rhizosphere soil at different intervals							
Treatment	7 days	15 days	30 days	45 days	60 days			
M. phaseolina	5.12 ± 0.21	5.97 ± 0.19	5.82 ± 0.20	5.17 ± 0.17	5.20 ± 0.11			
Bradyrhizobium AHR-2	6.91 ± 0.19	7.12 ± 0.15	6.67 ± 0.13	5.63 ± 0.06	5.47 ± 0.13			
Bradyrhizobium AHR-5	6.72 ± 0.20	6.90 ± 0.14	6.90 ± 0.13	5.42 ± 0.17	5.19 ± 0.11			
Bradyrhizobium AHR-6	6.80 ± 0.17	6.92 ± 0.15	6.49 ± 0.11	5.51 ± 0.11	5.22 ± 0.13			
Bradyrhizobium AHR-2 + M. phaseolina	6.81 ± 0.16	7.01 ± 0.11	6.77 ± 0.15	5.52 ± 0.09	5.37 ± 0.08			
Bradyrhizobium AHR-5 + M. phaseolina	6.12 ± 0.21	6.62 ± 0.17	6.13 ± 0.13	5.39 ± 0.17	5.09 ± 0.10			
Bradyrhizobium AHR-6 + M. phaseolina	6.67 ± 0.19	6.73 ± 0.13	5.14 ± 0.12	5.41 ± 0.11	5.11 ± 0.11			

Values are mean of five replicates; **, Significant at 0.01 level of LSD compared to M. phaseolina treatment; *, Significant at 0.05 level of LSD compared to M. phaseolina treatment.

lation, nodule weight and weight per nodule. Another advantage of working with *Bradyrhizobium* strain is the availability of better technical knowledge of inoculum production and application.

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Variability in response of *Helicoverpa* armigera males from different locations in India to varying blends of female sex pheromone suggests male sex pheromone response polymorphism

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Field trials were conducted at various locations in India to assess sex pheromone response of *Helicoverpa armigera* males to varying blends of its two sex pheromone components. In the pheromone septa that were used to bait the pheromone traps varying blends were impregnated and the ratio of Z-9: hexadecenal to Z-11: hexadecenal in them varied from 0:100 to 15:85. Results indicated geographical variation in response of males to varying blends of the two sex pheromone components, suggesting male sex pheromone response polymorphism.

THE phenomenon of polymorphism is exhibited in insects in various ways such as variation in forms, castes, phases, colours, etc. Among insects belonging to order Lepidoptera, pheromone polymorphism, particularly with respect to male sex pheromone response specificity, has been reported and in some cases is associated with insects having variation in host plants and/or in habitat environment¹⁻⁶. Since the American bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is polyphagous and also exists in varying environmental conditions, it was of interest to investigate the existence of geographical variation in male sex pheromone response variability in the species.

The sex pheromone of H. armigera consists of two major components – Z-9: hexadecenal and Z-11: hexadecenal^{7,8} Kehat et al.⁹ reported that in Israel, there was no significant difference in response of males to blends containing between 1 and 10% of Z-9: hexadecenal. While confirming this under Indian conditions, we enlarged the range to blends containing between 0 and 15% of Z-9: hexadecenal. (A blend designated as 0%

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