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# SOYBEAN RESEARCH

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# Differentiation Abilities of Callus Induced from Diverse Explants of Soybean (*Glycine max* L. Merrill)

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**ABSTRACT**

*To search out the explants with higher regeneration potential- immature embryonic axes, immature and mature cotyledons and hypocotyls of eleven cultivars of Glycine max were cultured on MS basal medium with 10 different combinations of auxins and cytokinins in varying concentrations. The culture medium MS3BN (MS + 3.0 mg/l BAP + 0.5 mg/l NAA + 30.0 g/l sucrose + 8 g/l agar) showed better response. Mature cotyledon explants exhibited higher in vitro morphogenesis closely followed by hypocotyl. For various explant cultures, cultivar JS 90-41 was found the most responsive except for mature cotyledon. Phenotypically normal plants were obtained after hardening in green house.*

**Key words:** *Glycine max*, embryonic axes, immature and mature cotyledon, hypocotyl, callus, *in vitro*, morphogenesis.

Soybean (*Glycine max* L. Merrill) is an imperative source of high quality protein and oil. The new gear of biotechnology will to realm us to speed up the development of improved cultivars with increased resistance to diseases and insects and greater tolerance to various abiotic stresses of cultivated soybean.

Although, all plant cells are derived from the fertilized egg cell and contain identical information, callus derived from somatic cells varies in competence to express totipotency i.e. their genetic ability to produce plants. The way in which a callus forms a new plant *in vitro* is capricious, and varied with explants as well as with presence of relative concentrations of auxin/ cytokinin ratio in to the culture medium. In soybean, various explants have been used efficiently to produce regenerable cultures via organogenesis and/or embryogenesis such as embryos (Lazzeri *et al*. 1987 a, and b, Hammatt and Davey 1987, Komatsuda and Ohyama 1988, Yeh 1989, Zhou *et al*. 1990, Komatsuda and Kao 1990, Amer 1992, Nawaracala *et al*. 1996, Hazel *et al*. 1998, Tripathi and Tiwari 2004b), cotyledons (Ancelet *et al*. 1988, Ferreira *et al*. 1990, Kothari *et al*. 1991, Lamseejan *et al*. 1993, Bodanse-Zanettini *et al*. 1993, Thome *et al*. 1995, Fu *et al*. 1995, Nawaracala and Konieczny 1996, Santarem *et al*. 1997, Tripathi and Tiwari 2003b; 2004a) and hypocotyls and epicotyls (Wright *et al*. 1987, Shu and Yeh 1988, Li *et al*. 1989, Kadlec *et al*. 1991, Cristea and Cachita-Cosima 1992, Rajasekran and Pellow 1997, Tripathi and Tiwari 2003a).

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Prior to the transformation of *Glycine max* a desirable gene(s) and local responding cultivars need to be identified. For transformation purposes, it is fundamental to formulate an efficient and reproducible protocol for plant regeneration. In view of this, an experiment was conducted to select the most responding explant, cultivar and cytokinin-to-auxin ratio exhibiting *in vitro* morphogenesis at higher frequency.

## MATERIALS AND METHODS

Seeds of eleven established cultivars of *Glycine max* (Table 2) were obtained from All India Coordinated Research Project on Soybean, Jawaharlal Nehru Agricultural University, Jabalpur. In preliminary experiment, ten different fortifications of MS (Murashige and Skoog 1962) culture media were prepared from ready-made powder (procured from HiMedia®, Mumbai, India) by supplementing different types of growth regulators in varying concentrations. On the basis of *in vitro* response, an auxin in lower concentration was used in combination with a higher concentration of cytokinin denoted as MS3BN (3.0 mg/l BAP and 0.5 mg/l NAA, MS macro- and micro- nutrients, vitamins, 30.0 g/l sucrose and 0.8% agar) for final experiment. Culture medium was autoclaved at 121°C under 15 psi pressures for 20 min after adjusting the pH to 5.6 ± 0.1 with 1N NaOH/HCl.

Immature pods were harvested from young field grown plants for the isolation of immature embryonic axis and cotyledons and surface sterilized with 70 percent (v/v) ethanol for 1 minute followed by a 5-minute treatment with 1 percent (w/v) mercuric chloride. Then pods were rinsed in sterile double distilled water under asepsis thrice. The explants mature cotyledons and hypocotyls were obtained from 4 days old germinated seeds. For this purpose, mature seeds were surface sterilized same as immature pods, except a different concentration (0.2%) and duration (10 min) of $HgCl_2$ was applied. Surface sterilized seeds were inoculated in culture tubes containing agar gelled water (8.0 g/l agar) under diffused luminance of 16 μ mol $m^{-2}$ $s^{-1}$ provided with white fluorescent lamps. Eight to 10 immature embryonic axes (2-8 mm, 15-20 days post-anthesis), 6-8 immature cotyledons (3-4 week post-anthesis) and 7-8 hypocotyls were plated on the culture medium in 100 x 17 mm glass petridishes. The cultured petridishes were sealed with Para film® and incubated under complete darkness at 25±2°C for a week after which incubates were subjected to photoperiod regime of 30μ mol $m^{-2}$ $s^{-1}$ luminance provided by white fluorescent lamps for 12 hours.

The calli were transferred on plant growth regulators free MS medium for morphogenesis. The MS medium for plant regeneration was fortified with 0.4 mg/l BAP and 0.4 mg/l NAA and the sucrose concentration was alleviated to 20.0 g/l. Where necessary, the *in vitro* shoots were subsequently transferred to a rooting medium (MS medium supplemented with 1.0 mg/l IBA and further reduced level of 15.0 g/l sucrose). Reduced level of sucrose was supplied in to regeneration and rooting media as described by (Tripathi and Tiwari 2003 a, b; Tripathi and Tiwari 2004 a, b). The competence of cultivars for *in vitro* shoot proliferation was evaluated 4 weeks after the transfer of the respective calli to the plantlet regeneration medium. Each callus body was counted as one, irrespective of the number of shoots initiated. Plural shoot induction was not counted separately at this stage and shooting efficiency was defined as the percentage of explants from which shoot(s) emerged. The experiment was laid out in factorial completely randomized design, with two factors, first cultivar at eleven levels and second explant at four levels with three replications. The arc-sine transformation was made before the analysis of data, since all data were in percentage. The data were analyzed as per method suggested by Snedecor and Cochran (1967).

The roots of plantlets were rinsed in sterile lukewarm water to wash-off the agar. The plantlets were potted in plastic root trainers containing a 3:1 mixture of autoclaved sand and soil. Root trainers with plants were acclimatized in a glass house under 30±2°C and 60±5 percent RH for 30 days.

**RESULTS AND DISCUSSION**

To establish successful plant tissue cultures and to select suitable explants for regeneration it is essential to have full knowledge of natural propagation system of plants (Hartman and Kester 1986). In soybean, most of the tissue culture experiments have been conducted on young leaves, hypocotyls, epicotyls and cotyledons obtained from germinating seeds or seedlings developed under lab/controlled conditions. On the other hand, immature embryos and cotyledons may be collected from plants grown under field or green house conditions. However, growing conditions of donor plants for *in vitro* culture, affect the plant regeneration efficacy up to a great extent.

During present investigation, immature embryonic axis, immature and mature cotyledon and hypocotyl explants of eleven *Glycine max* cultivars were cultured on fortified MS media to search out the explants with higher regeneration potential. In the first week of culture, the explants enlarged but no callus proliferation was evident up until the second week when a majority of the incubated tissues exhibited signs of callusing. In cultured immature embryonic axis, callus induction was usually observed from the whole explant. In cotyledons, callus proliferation started usually from the edges. In hypocotyl segments, profuse callus growth started from the both cut ends and spread towards the middle portion. The callus varied characteristically not only with the genotypes but also with the explants and their distinct phenotypes viz. wet, rough, hard, fragile, dense and glossy, reflected diversity in the developmental potentials were observed. This is accordance to findings of Gai and Guo (1996) who stated that different explants exhibited different callus types, induction frequencies and growth rates.

The performance of each cultivar in terms of callus induction was recorded after five weeks of incubation. Based on their appearance, four main types of calli were observed; here in after they were classified in to three main categories. (A) Embryogenic: a- compact dark green with few or many bead like structures, b- compact light green in colour, with few or many dark green bead like structures, and some times covered with a thin layer of white loose callus; (B) Organogenic: light green with dense and glossy appearance, and (C) Undifferentiated: cream in colour, soft and friable in texture. The data of two categories (embryogenic and organogenic calli) was grouped together to form the 'morphogenic callus' group.

In the case of embryogenic calli embryo like structures initiation started in 2 weeks from inoculation. However, in some incubates this was delayed up to 4 weeks. The embryo like structures were globular or beadlike, with irregular boundaries, usually appeared in clusters. In a few cases, both types of growth patterns (embryogenic as well as organogenic) were observed on the same callus. Morphogenic efficiency was defined as the percentage of explants in which such callus proliferated. In the preliminary experiment, callus texture and morphology was determined by the type and pace of exogenous growth regulators supplemented into culture medium. Explants cultured on medium MS3BN (higher proportion of a cytokinin with lower concentration of an auxin) initiated calli of embryogenic ‘a’ and ‘b’ types and regenerated shoots as well as roots. Culture media containing a higher concentration of an auxin with lower concentration of a cytokinin in majority promoted the formation of ‘B’ type organogenic

calli and preferred rhizogenesis instead of gamogenesis. The presence of an auxin alone in culture medium supported profuse callus growth of 'C' type. Such 'C' type calli after transfer to the medium without growth regulators not exhibited morphogenesis at full potential as compared to cultured on medium MS3BN. This revealed that higher auxin concentrations is not appropriate for the formation of embryogenic 'a' and 'b' types calli. On the other hand, a combination of a higher proportion of cytokinin to auxin is necessary for increased morphogenesis for the most of the explant cultures.

The analysis of variance presented in Table 2 clearly indicated the presence of considerable amount of variations among the cultivars, explants as well as cultivar x explant interactions in terms of overall callulogenesis and morphogenesis at 1 percent probability level. For callus induction, explant mature cotyledon (89.61%) was significantly superior to hypocotyl (87.43%) followed by immature embryonic axis (77.94%) and immature cotyledons (76.48%). Maximum morphogenic calli formation was recorded from mature cotyledon (56.55%) intimately followed by hypocotyl (56.04%). In com-

**Table 1.** ***In vitro*** **response of soybean on different culture media**

| Culture Medium | Growth regulators (mg/l) | | | Culture response * | | | |
|---|---|---|---|---|---|---|---|
| | BA | NAA | Others | Callus initiation | Friable Calli | Morpho-genic calli | Rhizo-genesis |
| MS3BN | 3.0 | 0.5 | - | + + + | - | + + + | + |
| MSNB | 1.0 | 1.0 | - | ++ | + | + | + |
| MS2NB | 0.2 | 2.0 | - | ++ | ++ | + | + |
| MSPB | 2.5 | - | 3.0 PCPA | +++ | + | ++ | - |
| MS3PB | 0.5 | - | 3.0 PCPA | +++ | ++ | + | - |
| MS10PB | 0.5 | - | 10.0 PCPA | +++ | ++ | + | - |
| MSIB | 0.5 | - | 0.2 IAA | + | + | + | + |
| MS5P | - | - | 5.0 PCPA | +++ | ++ | + | - |
| MS8N | - | 8.0 | - | +++ | ++ | + | - |
| MS30D | - | - | 30.0 2,4-D | +++ | ++ | - | - |

** High >60% (+++), moderate 40-60% (+ +), low <40% (+)*

parison, two other explants i.e. immature embryonic axis and immature cotyledon initiated such type calli in lower frequencies. In this study, higher *in vitro* response exhibited by mature cotyledons and hypocotyl (obtained from germinated seeds under laboratory conditions), followed by immature embryonic axes and cotyledons (obtained from immature pods grown under field conditions). This is in accordance to the findings of Kumar and Kumar (1996) who stated that the explants collected from green house grown plants (more elongated and etiolated) regenerated more readily *in vitro* as compared to those collected from out side. Different explants of a single genotype do not responded identically in cultures, most likely due to varying gradients of endogenous hormone levels. Single explants collected from same source

**Table 2. Callus inducing and morphogenic calli forming explants cultured on MS3BN medium**

| Genotypes ▼ Explants► | Callus induction (%) | | | | | Morphogenic calli (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Immature embryonic axis | Immature cotyledon | Mature cotyledon | Hypocotyl | **Mean** | Immature embryonic axis | Immature cotyledon | Mature cotyledon | Hypocotyl | **Mean** |
| Bragg | 75.46 | 84.61 | 96.42 | 91.10 | **86.89** | 37.22 | 41.48 | 77.34 | 48.93 | **51.24** |
| JS 72-280 | 82.77 | 85.42 | 85.78 | 83.28 | **84.31** | 41.82 | 44.34 | 40.65 | 49.00 | **43.92** |
| JS 72-44 | 63.88 | 53.12 | 88.24 | 84.19 | **72.35** | 30.24 | 25.79 | 83.45 | 41.40 | **45.22** |
| JS 75-46 | 74.12 | 71.79 | 91.55 | 93.06 | **82.63** | 36.55 | 37.26 | 38.33 | 68.96 | **45.27** |
| JS 80-21 | 82.81 | 96.25 | 86.37 | 91.54 | **89.24** | 51.07 | 49.96 | 44.09 | 61.63 | **51.68** |
| JS 90-41 | 88.07 | 93.90 | 92.79 | 93.55 | **92.07** | 49.62 | 41.88 | 60.19 | 81.03 | **58.18** |
| JS 335 | 77.69 | 67.37 | 95.17 | 81.25 | **80.37** | 36.15 | 34.55 | 53.88 | 69.99 | **41.14** |
| MACS 13 | 90.27 | 71.08 | 94.51 | 93.01 | **87.21** | 49.11 | 33.96 | 65.14 | 57.11 | **51.33** |
| NRC 2 | 68.62 | 77.32 | 73.11 | 85.61 | **76.16** | 34.52 | 36.24 | 22.36 | 65.20 | **39.58** |
| Panjab 1 | 82.71 | 53.27 | 92.05 | 79.22 | **76.81** | 43.73 | 31.55 | 84.74 | 41.10 | **50.28** |
| PK 472 | 71.00 | 87.13 | 89.78 | 86.00 | **83.47** | 33.64 | 49.99 | 51.92 | 32.15 | **39.67** |
| **Mean** | **77.94** | **76.48** | **89.78** | **87.43** | | **40.33** | **38.81** | **56.55** | **56.04** | |
| **CD (P = 0.05)** | | | | | | | | | | |
| Genotypes | | | | | **2.41** | | | | | **1.43** |
| Explants | | | | | **1.45** | | | | | **0.86** |
| Genotype x Explants | | | | | **4.82** | | | | | **2.86** |

**Table 3. Number of plants regenerated on regeneration medium from morphogenic calli of eleven soybean genotypes raised from four diverse explants**

| Genotype▼ Explant ► | Plantlets regenerated (%) | | | | |
|---|---|---|---|---|---|
| | **Immature embryonic axis** | **Immature cotyledon** | **Mature cotyledon** | **Hypocotyl** | **Total** |
| Bragg | 31.00 | 26.30 | 54.30 | 28.20 | **34.95** |
| JS 72-280 | 28.00 | 22.10 | 14.60 | 36.50 | **25.30** |
| JS 72-44 | 11.40 | 18.60 | 78.60 | 22.20 | **32.70** |
| JS 75-46 | 22.80 | 14.40 | 24.50 | 31.30 | **23.25** |
| JS 80-21 | 32.30 | 41.50 | 21.20 | 62.30 | **39.32** |
| JS 90-41 | 78.90 | 52.30 | 22.40 | 88.60 | **60.55** |
| JS 335 | 32.40 | 16.30 | 20.20 | 43.20 | **28.02** |
| MACS 13 | 36.40 | 23.40 | 44.50 | 14.30 | **29.65** |
| NRC 2 | 20.60 | 14.80 | 26.60 | 61.20 | **30.80** |
| Punjab 1 | 54.40 | 22.50 | 100.60 | 44.10 | **55.40** |
| PK 472 | 18.60 | 21.20 | 17.30 | 22.20 | **19.82** |
| **Total** | **33.34** | **24.85** | **38.61** | **41.28** | |

behaved differently in culture depending upon size and location of donor plant. Influence of various factors on *in vitro* response indicates that something within explants is as critical for a given response as its genotype.

In terms of the varietal response to *in vitro* culture, three of the eleven cultivars were found more responsive for the callus induction. Cultivars JS 90-41 (92.07%) proved superior over JS 80-21 (89.24%), MACS 13 (87.21%), Bragg (86.89%) and JS 72-280 (84.31%). Remaining genotypes were performed in similar manner and JS 72-44 (72.34%) proved to be the lowest performer. Proportion of calli resulting in morphogenesis was highest in cultivars JS 90-41 (58.18%) followed by correspondingly capable group of four cultivars JS.80-21 (51.68%), MACS 13 (51.33%), Bragg (51.24%) and Panjab 1 (50.28%). Lowest result was exhibited by NRC 2 (39.58%). It seems reasonable to conclude that variation obtained for *in vitro* response resulted from the genetic differences among genotypes, since; explants obtained from single source were distributed randomly to culture media. Many of the genetic differences could be circumvented by growing the source plants under optimal conditions and by varying nutrients and growth regulators in the culture medium. In soybean, earlier, genotypic differences have been recorded for cultured embryos (Hammat and Davey 1987, Komatsuda and Ohyama 1988, Nawracala *et al*. 1996, Tripathi and Tiwari 2004b), and cotyledon culture (Parrott *et al*. 1989, Ferreira *et al*. 1990, Komatsuda 1990, Kothari *et al*. 1991, Komatsuda *et al*. 1991, Bailey *et al*. 1993, Bodanse-Znettini *et al*. 1993, Thome *et al*. 1995, Li and Grabau 1996, Nawracala and Konieczny 1996, Tripathi and Tiwari 2003a; 2004b).

*In vitro* level, the interaction between the cultivar and the explant was also evident. Explants dependent response of the cultivars for

plant regeneration is presented in Table 3. Cultivar JS 90-41 exhibited higher plant regeneration from all explants except mature cotyledon, where, Punjab 1 followed by JS 72-44 regenerated higher plantlets. Among four explants, maximum plantlets regenerated from hypocotyl explants followed by mature cotyledon, immature embryonic axis and immature cotyledon. This is conflict to findings of Shu and Yeh (1988) as they obtained higher *in vitro* plant regeneration from cultured cotyledons followed by shoot tips and hypocotyls. During present investigation, cultivar found most suitable for one explant culture may respond poorly to other explant culture, even on a similar medium. This can be explained by example of genotype JS 90-41, which regenerated higher plants from immature embryonic axis, immature cotyledon and hypocotyl cultures whereas, from mature cotyledon, plant regeneration frequency was quite low as compared to other cultivars. This may be possible due to presence of varying endogenous growth regulator levels among different explants that mostly regulate the competence and incidence of morphogenesis. Furthermore, interaction between endogenous and exogenous growth regulators influences occurrence of morphogenesis (organogenesis and embryogenesis) up to a great extent.

The prevalence of embryogenesis in somatic cells has been found to be associated with cultures initiated from embryo explants rather than non-embryogenic and matured tissues. Embryogenic calli usually possess high regeneration potential. During present study explants hypocotyls and immature embryonic axis regenerated plantlets via embryogenesis rather than organogenesis, while explant mature cotyledon regenerated plantlets via both the pathways. Yeh (1989) observed callus, shoot and root formation in 10-14 DAP (days after pollination) embryos but not in younger embryos. Shoot and plantlet differentiation occurred in 15-20 DAP embryos while, embryos over 21 DAP gave rise to plantlets via organogenesis. In this experimentation 14 DAP immature embryos were taken for culture seeing as they mostly regenerated plantlets via embryogenesis.

The plants, after survival in the glasshouse conditions, were evaluated visually based on their appearance. Although, the traits were not scored quantitatively, the plants obtained from different explant cultures were phenotypically normal and true to the type. Present investigation clearly suggests that explants with higher regeneration potential should be used for crop improvement though unconventional means.

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# Flower Production and Abscission Rate in Relation to Yield and Yield Components in Soybean [*Glycine max* (L.) Merrill]

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**ABSTRACT**

*To investigate the extent of genetic variability for flowering traits viz. flower production, flower span (days), rate per day flower production and reproductive abscission (by taking difference between total flower production and matured pod developed) along with seed yield and other yield contributing traits, sixteen genotypes of soybean with different growth habits were used for this study. Grain yield per plant showed highly significant positive correlation with dry matter weight, number of pods per plant, seed yield efficiency and harvest index. Total flower production per plant showed highly significant positive association with flower production per day, number of flower dropped, number of pods per plant and number of seeds per pod. Reproductive abscission exhibited significant positive correlation with number of flowers dropped whereas; it showed significant negative correlation with number of pods per plant and dry matter weight. High to moderate broad sense heritability was reported for most of the characters viz. oil percent, total flower production, days to flowering, number of primary branches, number of pods per plant, protein percent and number of seeds per pod; whereas, abscission rate, harvest index and dry matter weight per plant exhibited low heritability. Jupiter, a genotype with indeterminate growth habit possessing highest grain yield i.e. 38.22 g per plant had lowest reproductive abscission rate (34.6%). Though, highest number of flower i.e. 462 was produced by PK 1241 (semi-determinate genotype), 317 flowers dropped, consequently the reproductive abscission rate was high i.e. 68.95 percent. Low to moderate reproductive abscission rate i.e. 53.9, 53.91, 54.63, and 59.61 percent was recorded in PK 1024, T 49, PK 262 and PK 1042, respectively. These genotypes possessing high flower production with low to moderate abscission rate could be considered for yield improvement in soybean breeding programme.*

**Key words:** soybean, flower production, abscission rate, yield components

Genetic variability in any crop is the essential prerequisite for initiation of breeding work, whereas, improvement is possible only when variability exist in the population of that species. Specially, the hereditary variation is of major interest to the plant breeders without which there could be no heritable plant improvement. The three main yield components in soybean can be

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described as pod number per unit area, seeds per pod and mean seed weight. Pod number is determined by the extent of flower production, abortion and abscission. The number of pods retained by soybean plant is an important yield determinant and perhaps, reducing reproductive abscission might increase yield. Reproductive abscission has been shown to be quite extensive for several soybean-cultivars. However, little information is available which describe abscission level and pattern in determinate and indeterminate soybeans. Van Sehaik and Probst (1958) reported total soybean reproductive abscission ranged from 32 to 81 percent and also observed that percentage of abscised pod was relatively constant whereas, variation in total abscission was due to differences in flower abscission. Selection for flowers per plant, reproductive abscission rate and pods per plant may increase the probability of combining useful trait in a single plant type. The present study was therefore, undertaken with the objective to obtain estimates of variability and heritability for flower production, reproductive abscission rate, seed yield and other related traits and determining correlations between the flowering traits, seed yield and other yield contributing traits.

## MATERIAL AND METHODS

The experimental material for the present study comprised of 8 determinate (PK 262, PK 1042, PK 1029, PK 317, PK 327, PK 1092, PK 1162 and PK 1024) and 8 semi-determinate/indeterminate (Kalitur, T 49, Ankur, JS 335, PK 416, Jupiter, PK 1241 and TS 1-3-5-3) popular varieties/breeding lines, which were grown in a randomized block design with 3 replications during *kharif* 1998. Each plot consisted of 3 rows, 4 meters long and spaced 60 cm apart, with spacing of 5 cm between plants in each row. Five randomly selected plants in the central row of each plot were tagged for recording the observations on days to flowering, flower production per day, total flower production, flower span (days), reproductive abscission rate per plant (%), number of flowers dropped, number of primary branches, number of pods per plant, number of seeds per pod, dry matter weight per plant, harvest index, seed yield efficiency, grain yield per plant, protein and oil content. The estimates of genotypic and phenotypic coefficient of variation (GCV and PCV), broad scenes heritability and expected genetic gain were worked out by the method of Burton and De vane (1953), Allard (1960), respectively. The correlations between all the characters under study at genotypic, phenotypic and environmental levels were estimated according to the method given by Searle (1961).

## RESULTS AND DISCUSSION

The differences between PCV and GCV estimate for characters viz. days to flowering, flower span, number of primary branches, number of pods per plant, number of seed per pod, protein percent, oil percent and grain yield per plant were comparatively low, suggesting that these characters were relatively less influenced by the environment.

High value of GCV was reported (Table 1) for number of flower drop (29.42 %) followed by total flowers per plant (28.56 %) and grain yield per plant (27.92 %). Existing sufficient genetic variability in the present material for these flowering traits provides an opportunity to execute effective selection criteria for their improvement. Flower span, abscission rate, number of seeds per pod, oil percent and protein percent had quite low GCV, whereas, number of pods per plant, seed yield efficiency, days to flower, flower production per day and number of primary branches per plant had moderate GCV suggest that variability in these traits may be created further through breeding to make the selection more effective. In general, PCV estimates were higher than corresponding GCV and ECV value for all the characters under study.

**Table 1. Estimation of genetic parameter for fifteen characters in soybean**

| Characters | PCV (%) | GCV (%) | ECV (%) | Heritability | Genetic advance (%) |
|---|---|---|---|---|---|
| Days to flowering | 13.96 | 12.6 | 6.01 | 81.45 | 10.03 |
| Flower production per day | 18.19 | 13.97 | 11.65 | 58.97 | 2.04 |
| Total no. of flowers/plant | 28.73 | 28.56 | 3.05 | 80.03 | 15.31 |
| Flower span (days) | 10.89 | 8.50 | 6.80 | 60.94 | 3.67 |
| Number of flower dropped | 41.98 | 29.42 | 14.43 | 88.17 | 12.96 |
| Number of primary branches | 16.06 | 14.35 | 7.22 | 79.79 | 24.43 |
| Number of pods per plant | 13.67 | 11.68 | 7.08 | 73.12 | 22.28 |
| Number of seeds per pod | 8.30 | 6.97 | 4.51 | 7.48 | 0.26 |
| Dry matter per plant | 13.24 | 9.00 | 9.71 | 49.93 | 11.39 |
| Harvest index | 13.36 | 9.44 | 9.45 | 46.23 | 3.99 |
| Seed yield efficiency | 15.20 | 12.21 | 9.61 | 63.69 | 0.10 |
| Abscission rate (%) | 11.93 | 7.58 | 9.21 | 40.41 | 2.59 |
| Protein (%) | 1.93 | 1.65 | 1.00 | 72.88 | 1.13 |
| Oil (%) | 5.00 | 4.87 | 1.16 | 94.58 | 1.95 |
| Grain yield per plant (g) | 30.18 | 27.92 | 11.45 | 65.59 | 11.76 |

The estimates of PCV and GCV for days to flowering, total flowers, flower span, number of primary branches, number of seeds per pod, protein and oil percent were more or less similar indicating that these character were comparatively stable. Similar to the present study, high estimate of GCV and PCV for seed yield were reported in soybean by Kumar and Haque (1973), Chandra *et al.,* (1975) and Singh *et. al.* (1981). A low estimate of coefficient of variability for days to flowering was also reported by Singh *et al.,* (1981). Most of the traits included in this investigation were considered highly heritable as they have shown to be associated with high broad sense heritability viz., oil percent (94.58 %), number of flower dropped (88.17 %), days to flowering (81.45 %), total flower (80.03 %), whereas moderately high estimates were observed for number of primary branches (79.79 %), number of pods per plant (73.12 %), protein percent (72.83 %), number of seed per pod (70.43 %), grain yields per plant (65.59 %), seed yield efficiency (63.69 %), flower span (60.94 %) and flower production per day (58.97 %), Surlan-Momirovic (1987) and Sharma (1980) also observed similar results in soybean.

Simple correlations (Table 2) indicated that grain yield per plant had highly significant positive correlation with dry matter weight (0.712), seed yield efficiency (0.485), number of pods per plant (0.474) and non-significant positive correlation with days to flowering (0.169), flower production per day (0.153), total flower (0.305), flower span (0.281), harvest index (0.452) and oil percent (0.181), however, grain yield was negatively correlated with number of flower drop (-0.050), abscission rate (- 0.404) and protein

percent (- 0.399). Reproductive abscission rate per plant (%) showed highly significant positive relationship with number of flower dropped (0.629) whereas, significant negative association was reported for number of pods per plant (- 0.704) and for dry matter (- 0.586) whereas, correlation with other traits viz. flower production per day, harvest index and seed yield efficiency was positive but non-significant. Similar association with reproductive abscission was also reported by Gai *et al.* (1984) in soybean. Harvest index showed highly significant positive correlation with seed yield efficiency (0.946) and dry matter weight exhibited highly significant/significant positive inter character association with number of pods per plant (0.685), number of primary branches per plant (0.548), whereas this relationship was positive but non-significant with days to flowering, total flower production and flower span and number of pods per plant (0.562). Similarly significant positive correlation was observed between number of pods per plant with total flower per plant (0.562), total flower per with flower production per day (0.934). These results are in accordance with findings documented by Jadhav *et al.,* (1995), Mehetre *et al.,* (1997) and Tong (1986) observed in soybean. Heindl and Brun (1984) reported that pods per node had more impact on seed yield than seed weight, whereas, flower abscission was the main determinant of pods per node. Sharma (1980) suggested that flower production, raceme length, flower per raceme and pod types were heritable traits in soybean crosses, whereas flower abortion was a more stable genotypic characteristics than flower per raceme. Moreover, expectations to the close association that has previously been reported between high flower abortion and high number of flower per plant have been observed in widely differing genotypes.

Jupiter, a genotype with indeterminate growth habit, was reported to produce highest grain yield. Whereas, reproductive abscission rate was lowest i.e. 34.60 percent, it produced fairly large number of flowers (309) but about 3 % flowers (101) dropped. Another genotype, PK 1241 (semi-determinate) produced highest number of flowers per plant i.e. 462, out of which 317 flowers were aborted; consequently the reproductive abscission rate was reported to be high 68.95 percent. Low to fairly high abscission rate i.e. 55.9, 53.91, 54.63, 59.61 and 65.44 was reported for PK 1024, T-49, PK 262, PK 416 and PK 1042, respectively, which gave numerically high yield over the general mean (22.10 g/plant). Maximum number of flower abscised in case of PK 1241 (317 flowers) followed by PK 317 and PK 1042 (293 and 259 flowers, respectively).

A perusal of table 3 based on 16 genotypes (8 determinate and 8 indeterminate) indicated that on an average a soybean plant starts blooming after 43 days from planting and produced 283 flowers at the rate of 10 flowers per day within 23 days of flower span out of which approximate 170 flowers aborted and remaining 111 flower got converted into pods indicating abscission rate of approximately 60 %. Whereas, the same plant by attaining 67.46 g dry matter converted into 22.10 g grain yield with harvested index of 0.34.

In an earlier study on soybean, Wiebold *et al.,* (1981) reported that cultivars differed in number of flowers, young pods and pods produced per plant. Number of flowers produced per plant ranged from 170 to 332 and averaged 232 and pods at harvest ranged from 41-90 and averaged 57 per plant, whereas, percentage total abscission ranged from 67 to 82 percent with an average 75 percent. Similarly, Brevedan *et al.,* (1978) observed that abscission of 54 and 57 percent for a cultivar of soybean grown in the field and green house, respectively. From this investigation it is concluded that in addition to yield and yield components, sufficient genetic variation exists in the flowering traits *viz.*, total

**Table 2. Simple correlation coefficient among yield and yield contributing traits in soybean**

| Characters | Days to flowering | Flower prodn./ day | Total flower/ plant | Flower span (day) | No. of flower drop | No. of primary branches/ plant | No. of pods/ Plant | No. of seeds/ pod | Dry matter (g) | Harvest index | Seed yield efficiency | Abscission rate (%) | Protein (%) | Oil (%) | Grain yield/ plant(g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days to flowering | | -0.032 | -0.044 | -0.145 | -0.306 | -0.269 | 0.216 | -0.117 | 0.218 | -0.168 | -0.190 | -0.430 | 0.168 | 0.041 | 0.169 |
| Flower prodn./day | | | 0.934** | -0.672** | 0.883** | 0.189 | 0.119 | 0.588* | 0.148 | 0.028 | -0.035 | 0.281 | 0.117 | 0.055 | 0.153 |
| Total flower/plant | | | | -0.419 | 0.819** | 0.397 | 0.562* | 0.525* | 0.434 | -0.097 | -0.155 | 0.143 | -0.021 | -0.080 | 0.305 |
| Flower span (day) | | | | | -0.522** | 0.170 | -0.147 | -0.132 | 0.336 | 0.027 | 0.174 | -0.235 | -0.372 | -0.440 | 0.281 |
| No. of flower drop | | | | | | 0.164 | 0.087 | 0.460 | -0.095 | 0.125 | 0.072 | 0.629** | 0.044 | 0.080 | -0.050 |
| No. of primary branches/ plant | | | | | | | 0.330 | 0.366 | 0.548* | -0.325 | -0.418 | -0.092 | -0.007 | -0.462 | 0.110 |
| No. of pods/Plant | | | | | | | | 0.185 | 0.685** | -0.255 | -0.277 | -0.704** | 0.127 | -0.071 | 0.474* |
| No. of seeds/pod | | | | | | | | | 0.133 | -0.141 | -0.204 | 0.183 | 0.348 | -0.040 | 0.035 |
| Dry matter (g) | | | | | | | | | | -0.264 | -0.233 | -0.586* | -0.199 | -0.398 | 0.712** |
| Harvest index | | | | | | | | | | | 0.946** | 0.277 | -0.320 | 0.130 | 0.452 |
| Seed yield efficiency | | | | | | | | | | | | 0.256 | -0.339 | 0.092 | 0.485* |
| Abscission rate (%) | | | | | | | | | | | | | 0.170 | 0.088 | -0.404 |
| Protein (%) | | | | | | | | | | | | | | -0.061 | -0.399 |
| Oil (%) | | | | | | | | | | | | | | | 0.181 |
| Grain yield/ plant (g) | | | | | | | | | | | | | | | |

** Significant at 5% level; ** Significant at 1% level*

**Table 3. Mean values for seed yield and other yield related characters**

| Name of genotypes | Days to flower-ing | Flower prodn./ day | Total flower/ plant | Flower span (day) | No. of flower drop | Primary branches/ plant | No. of pods/ plant | No. of seeds/ pod | Dry matter (g) | Harvest index | Seed yield efficiency | Abscission rate (%) | Protein (%) | Oil (%) | Grain yield/ plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Semi determinate* | | | | | | | | | | | | | | | |
| Kalitur | 53 | 7 | 205 | 28 | 120 | 6 | 83 | 2.12 | 43.44 | 0.35 | 0.56 | 59.02 | 40.00 | 19.60 | 15.60 |
| T-49 | 56 | 11 | 265 | 25 | 143 | 9 | 121 | 2.17 | 70.33 | 0.35 | 0.54 | 53.91 | 39.50 | 20.00 | 24.81 |
| Ankur | 42 | 5 | 190 | 35 | 125 | 7 | 64 | 2.02 | 62.97 | 0.28 | 0.40 | 65.60 | 39.00 | 20.50 | 17.91 |
| JS 335 | 43 | 12 | 269 | 22 | 198 | 10 | 70 | 2.35 | 40.67 | 0.41 | 0.72 | 73.80 | 39.44 | 20.61 | 17.15 |
| PK 416 | 39 | 8 | 219 | 27 | 131 | 8 | 87 | 2.17 | 52.23 | 0.44 | 0.80 | 59.61 | 38.00 | 21.75 | 22.61 |
| Jupiter | 49 | 11 | 309 | 29 | 101 | 10 | 203 | 2.21 | 102.22 | 0.37 | 0.61 | 34.60 | 39.03 | 18.75 | 38.22 |
| PK 1241 | 44 | 22 | 462 | 21 | 317 | 9 | 143 | 2.67 | 81.67 | 0.37 | 0.61 | 68.95 | 39.50 | 19.61 | 31.12 |
| TS 1-3-5-3 | 44 | 8 | 299 | 37 | 204 | 12 | 90 | 2.20 | 104.22 | 0.17 | 0.20 | 67.32 | 38.76 | 18.83 | 18.16 |
| Mean | 41 | 10 | 277 | 28 | 167 | 9 | 108 | 2.23 | 69.22 | 0.34 | 0.55 | 60.25 | 39.15 | 19.96 | 23.20 |
| *Determinate* | | | | | | | | | | | | | | | |
| PK 262 | 43 | 8 | 266 | 32 | 139 | 9 | 119 | 2.08 | 80.44 | 0.31 | 0.45 | 54.63 | 38.50 | 21.03 | 25.01 |
| PK 1042 | 41 | 16 | 411 | 25 | 259 | 10 | 141 | 2.15 | 88.00 | 0.33 | 0.50 | 65.44 | 39.03 | 21.00 | 29.53 |
| PK 1029 | 40 | 7 | 208 | 31 | 103 | 9 | 104 | 2.18 | 65.11 | 0.31 | 0.47 | 49.70 | 38.00 | 20.73 | 20.64 |
| PK 317 | 35 | 14 | 392 | 29 | 293 | 9 | 97 | 2.14 | 40.75 | 0.51 | 0.82 | 75.07 | 38.50 | 18.70 | 18.50 |
| PK 327 | 40 | 12 | 298 | 26 | 197 | 9 | 100 | 2.24 | 45.00 | 0.36 | 0.57 | 66.46 | 39.67 | 21.16 | 16.42 |
| PK 1092 | 37 | 6 | 181 | 28 | 113 | 9 | 67 | 2.25 | 51.11 | 0.30 | 0.45 | 62.84 | 40.50 | 19.00 | 15.85 |
| PK 1162 | 40 | 10 | 264 | 26 | 122 | 9 | 140 | 2.13 | 70.66 | 0.28 | 0.38 | 46.48 | 38.76 | 19.10 | 19.87 |
| PK 1024 | 39 | 10 | 299 | 30 | 155 | 11 | 153 | 2.54 | 80.66 | 0.27 | 0.39 | 53.90 | 38.70 | 20.41 | 22.30 |
| Mean | 39 | 10 | 290 | 28 | 173 | 9 | 114 | 2.21 | 64.22 | 0.33 | 0.50 | 59.31 | 38.96 | 20.14 | 20.01 |
| GM | 43 | 10 | 283 | 28 | 170 | 9 | 111 | 2.22 | 67.46 | 0.34 | 0.53 | 59.83 | 39.05 | 20.05 | 22.10 |
| SEm± | 1.48 | 0.73 | 4.98 | 1.22 | 14.16 | 0.38 | 7.02 | 0.05 | 3.36 | 0.02 | 0.06 | 2.94 | 0.22 | 0.13 | 1.46 |
| **CD (%)** | **4.29** | **2.13** | **14.41** | **3.56** | **40.91** | **1.10** | **20.27** | **0.16** | **9.73** | **0.08** | **0.18** | **8.50** | **0.65** | **0.38** | **4.22** |
| CV (%) | 6.01 | 12.25 | 3.06 | 7.50 | 14.43 | 7.22 | 10.98 | 4.51 | 8.65 | 14.71 | 20.58 | 8.52 | 1.00 | 1.16 | 11.45 |

flower, number of flower dropped, per day flower production and reproductive abscission rate etc. From the supporting evidence it is suggested that particularly reproductive abscission rate and total flowers are considered as stable and heritable traits. Van Sehaik and Probst (1958) reported quantitative inheritance with dominance and complementary gene effects for long raceme, high flower number and flower abscission. Few genotypes viz., PK 1241, PK 1042, PK 317 and PK 1024 had high flower production, whereas Jupiter, PK 1162 and PK 1029 had low abscission rates. These genotypes could be considered to improve flowering traits particularly high flower production, which has positive association with yield. These genotypes may be considered in breeding programme to improve these flowering traits by utilizing them in crossing programme particularly for higher flower production in order to minimizing the reproductive abscission rate and appropriate breeding approaches to enhance the yield potential in soybean.

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# Interrelationship Among Lipoxygenase Isozymes, Polyunsaturated Fatty Acids and Trypsin Inhibitor During Seed Development in Soybean

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**ABSTRACT**

*Lipoxygenase isozymes, polyunsaturated fatty acids and trypsin inhibitor content were determined in four selected soybean varieties during seed development. A genotypic variation was observed for accumulation pattern of lipoxygenase isozymes and trypsin inhibitor, besides, significant levels of trypsin inhibitor in the early stage of soybean seed development. Linolenic acid was found to be maximum at 30 days after flowering and thereafter decreased continuously at varying rate in all the genotypes till maturity. Lipoxygenase isozymes levels were comparatively low in the early stage of development when linolenic acid was very high. A significant positive correlation of trypsin inhibitor content with lipoxygenase I as observed in present studies suggests a coordinated expression of these biological components during seed development in soybean.*

**Key words:** Soybean, lipoxygenase isozymes, polyunsaturated fatty acids, trypsin inhibitor, seed development

Lipoxygenase (Linoleate: oxygen oxidoreductase, EC 1.13.11.12) and trypsin inhibitor are considered undesirable components in soybean seeds (Rackis *et al.* 1979, Anderson-Hafferman *et al.* 1992). In general, normal soybean seed lipoxygenase (Lox) exists in three isozymic forms namely Lox-I, Lox II and Lox III (Axelrod *et al.* 1981) and constitutes about 1-2 percent of the proteins present in dry seeds (Kitamura 1984). These isozymes catalyse the hydroperoxidation of polyunsaturated fatty acids (PUFA), linoleic and linolenic acid, containing *cis cis 1,4 pentadiene* moiety and have been categorized into two classes. Class I lipoxygenase (Lox-I) is characterized by high pH optima of around 9.0 and formation of large amounts of 13-hydroperoxides while class II lipoxygenase (II + III) show pH optima of around 7.0 and formation of equal amounts of 9 and 13-hydroperoxides. The hydroperoxidation reaction catalysed by the lipoxygenase isozymes lead to the formation of volatile hexanal compounds

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which are responsible for the development off-flavour in soy products, the prime deterrent in their wider acceptability by Indian populace. Furthermore, soybean seed lipoxygenases have also been reported to cause seed deterioration (Bewley 1986). During storage and transport, due to slight mechanical or bacterial damage, PUFA in the membrane bound and storage lipids in seed become favorable substrates for lipoxygenase to catalyse oxidation. Free radicals generated in the process, set the chain reaction of oxidation of membrane lipids, ultimately disrupting membrane integrity (Vick and Zimmerman 1987). Trypsin inhibitor (TI), the protease inhibitor in soybean seeds, is responsible for reducing digestibility of proteins by inhibiting tryptic activity. Though TI is heat labile, the heat treatment insolubilizes the much–valued proteins (Anderson 1992) and cause loss of essential amino acids in soy proteins (Rios-Iriarte and Barnes 1996).

Globally, genotypic variability for Lox isozymes, PUFA and TI has been reported in mature soybeans (Marczy *et al.* 1995, Yang *et al.* 1999, Ishika *et al.* 2001). Recently, variability for these characters has been reported in mature seeds of among Indian genotypes (Kumar *et al.* 2001; 2002; 2003; Rani *et al.* 2004). The information on the developmental expression of Lox isozymes and their substrates, PUFA, and TI during seed growth is important to view the great potential of immature soybean pods for human consumption and to understand the interrelationship among these biological components during seed development. However, the reports focusing on the expression and interrelation of these biological components during seed development are few and scattered (Yao *et al.* 1983, Sekiya *et al.* 1986, Liu and Markakis 1987). Hence, the present study was undertaken to understand the interrelationship between lipoxygenases, PUFA and TI in developing seed tissues of four Indian soybean varieties.

## MATERIAL AND METHODS

Four commercial varieties of Indian soybean *viz.* JS 335, Pb 1, NRC 37 and Shilajeet were sown in 3 meters rows with a spacing of 45 cm in the experimental fields of National Research Centre for Soybean (ICAR), Indore on 27$^{th}$ June 2003. Sufficient number of plants was tagged in each variety on the day of flowering. Hand picking of green immature pods commenced from 30 days after flowering (daf) and continued till harvest maturity with an interval of 5 days. The seeds were removed from picked pods for further analyses.

### *Extraction and estimation of lipoxygenase isozymes*

For determination of lipoxygenase isozymes, fresh green seeds were ground using pestle and mortar in liquid nitrogen. The ground freeze-dried samples were defatted with petroleum ether and air dried to evaporate petroleum ether. The enzyme was extracted with soybean extract with 100 volumes of phosphate buffer (0.2 M, pH 6.8) by agitation in a micro tissue homogenizer for 20 minutes at 0-4$^0$C. The homogenized solution so obtained was centrifuged at 10, 000 rpm for 10 minutes at 4$^o$C. The supernatant so obtained was used as the crude extract for the assay of lipoxygense isozymes following the standard method (Axelrod *et al.* 1981). The reaction mixture for lipoxygenase-I consisted of crude extract as enzyme source, 2.8 ml of boric acid borax buffer (0.2 M, pH 9.0) and 10 mM sodium linoleate as a substrate. Lipoxygenase-II and III were analysed collectively with the reaction mixture consisting of crude extract as enzyme source, 0.2 M phosphate buffer (pH 6.8) and 10 mM sodium linoleate as a substrate. The change in absorb-

ance was recorded in Shimadzu UV-160 spectrophotometer at 234 nm. One unit of enzyme was taken as equivalent to the amount of enzyme that generated an increase in absorbance of 1.0 per minute due to conjugate diene in the enzymatic hydroperoxidation at 234 nm.

***Extraction and estimation of trypsin inhibitor (TI)***

One gram of fresh green pods was extracted in 50 ml of .01N NaOH for 4 hours with constant stirring at 125 rpm in an orbital shaker so as to keep the samples in suspension. The suspension so obtained was appropriately diluted so that 2 ml of the sample extract inhibited 40-60 percent of the trypsin used as a standard in the analysis. TI activity was determined by standard procedure (Kakade *et al*. 1974) as modified by Hammerstrand *et al.* (1981). Of the five test tubes taken, 2 ml aliquots of the diluted sample were added to the four test tubes. A fifth test tube was prepared for the trypsin standard by adding 2 ml of distilled water. To three of the four test tubes containing the sample extract, 2 ml of trypsin solution (prepared by dissolving 0.004 g of the trypsin in 200 ml of 0.001 N HCl) was added and were maintained at a constant temperature water bath 37$^{o}$C for 10 minutes. Five milliliters of benzoyl DL- arginine para nitroanilide hydrochloride (prepared by dissolving 0.08 g of benzoyl DL arginine paranitroanilide hydrochloride in 2 ml of dimethyl sulfoxide and diluted to 200 ml with 50 mM Tris buffer (pH 8.2) containing 20 mM calcium chloride and the contents were warmed to 37$^{0}$C) was rapidly added into each tube. The contents were stirred immediately on a vortex mixture and the tubes were placed in a water bath at 37$^{0}$C. The reaction was terminated after exactly 10 minutes by the rapid addition of 1 ml of 30 percent glacial acetic acid. The fourth tube containing sample extract (sample blank) was prepared by the same procedure except that the trypsin solution was added after the reaction was terminated by the addition of 30 percent glacial acetic acid. The absorbance of each solution was determined at 410 nm against the sample blank. Values obtained from each of the two sample extracts were subtracted from trypsin standard. These values were averaged and the trypsin content was determined as follows:

TI mg /g of defatted sample = Differential Absorbance x Dilution factor / 0.019 x 1000

Percent Inhibition = 100x differential absorbance / Absorbance of the standard

***Estimation of polyunsaturated fatty acids***

Oil was extracted from oven dried shelled seeds using petroleum ether (bp 40-60$^{o}$C). Methyl esters were prepared from the oil by interesterification in methanol using sodium methoxide as the catalyst (Luddy *et al*. 1968). Fatty acid methyl esters (FAMEs) prepared were separated and analyzed in gas liquid chromatograph (GLC), Shimadzu GC 17A, using capillary column with length and diameter of 30 meter and 0.32 millimeter, respectively. Oven temperature of the GLC was programmed at 140$^{o}$C for 3.6 minutes, and subsequently increased to 170$^{o}$C at the rate of 13.5$^{o}$C per minute and maintained for 3.8 minutes and finally to 182$^{o}$C at the rate of 5$^{o}$C per minute for obtaining best resolution of methyl esters. The temperatures of flame ionization detector (FID) and injector were maintained at 240$^{o}$C. Nitrogen, the carrier gas used, was maintained at a flow rate of 15 ml/minute with column pressure at 90 kpa. The peaks for individual FAMEs were identified by comparing the retention times with those of standard methyl esters (procured from Sigma, USA). The analysis was done in triplicate samples and the mean values were reported.

***Qualitative analysis of Kunitz inhibitor***

Kunitz inhibitor from developing seeds was extracted in Tris–Cl buffer (100 mM, pH 6.8) containing 0.23 M $CaCl_2$ and 5 mM phenyl methyl sulfonyl chloride (PMSF) following Kollipara *et al.,* (1991) and was resolved using non denaturing discontinuous polyacrylamide slab gel con-

sisting of 5 percent stacking gel (pH 6.8) and 10 percent resolving gel (pH 8.8) in BioRad Vertical Electrophoresis system (Laemmli 1970). The images were captured in Gene Genius Bio-imaging System of Syngene.

## RESULTS AND DISCUSSION

Data presented in Table 1 indicate the lipoxygenase isozymes, PUFA and TI content of four genotypes viz. JS 335, Pb 1, NRC37 and Shilajeet during seed development. Harvest maturity reached in 104, 108,100 and 105 days for Pb 1, JS 335, Shilajeet and NRC 37, respectively. Total lipoxygenase activity increased continuously from 30 daf (days after flowering) till maturity in Pb 1 and NRC 37, while in JS 335 and Shilajeet it increased till 50 daf and then decreased towards maturity. Lipoxygenase-I increased continuously from 30 daf to 50 daf and then decreased at maturity in all the genotypes except NRC 37 where it increased till maturity. Lipoxygenase-II+III activity peaked about 20 days before maturity in JS 335, while in case of Shilajeet and Pb 1 it reached maximum 10 days before maturity. In NRC 37, lipoxygenase-II + III activity kept on increasing up to maturity. Hildebrand and Hymowitz (1981) also reported that the profile of lipoxygeanse-I activity increased to maturity, while lipoxygenase-II+III activities became maximal between 5$^{th}$ and 20$^{th}$ days before maturity in the soybean genotypes investigated.

All the four genotypes exhibited significant levels of TI on dry weight basis at very early stage of development. Kunitz inhibitor, also known as SBTI-$A_2$, constitutes about 80 percent activity of TI activity in soybean (Moreira *et al.* 2004). The seeds samples picked at different developmental stages for different genotypes were tested electrophoretically for the presence of kunitz inhibitor band using kunitz inhibitor as marker protein. Kunitz inhibitor band was observed from 30 daf in all the four genotypes (Figs 1 and 2). This substantiated the presence of kunitz inhibitor at very early stage of seed formation. Accumulation pattern of TI content during seed development was different in different genotypes. TI activity on dry weight basis increased continuously from 30 daf till harvest maturity in Pb 1 and NRC 37, which is in consonance with earlier report (Liu and Markakis 1987). The TI activity in Shilajeet increased till 55 daf and thereafter, it decreased just before maturity as observed in variety Dare (Collins and Sanders 1976). JS 335 showed as high TI activity on dry weight basis at 30 daf as on maturity and thereafter, it fluctuated. TI activity decreased slightly at 35 daf, it remained stable till 45 daf and at 50 daf it showed higher value than at 45 daf. Thereafter it decreased before final increase at maturity. These genotypic differences for accumulation of TI content may be attributed to the presence of two types of proteases in developing soybeans. First category of protease catalyses the proteolytic processing of the precursor form to the mature form of kunitz inhibitor while another involves in proteolytic degradation of kunitz inhibitor (Mc Grain *et al*. 1992). The reduction in TI content in JS 335 and Shilajeet in late developmental stages of seed growth as observed in present study may be attributed to the higher activity of second type of protease.

Interestingly, among genotypes *viz.* JS 335 and NRC 37, which showed an increase in lipoxygenase-II+III activity between late reproductive stage and harvest maturity, TI content also increased during the corresponding period. Decrease in lipoxygenase-II+III activity in Shilajeet between late reproductive stage and harvest maturity corresponded to decrease in TI activity during the same period, however this type of trend was not observed in Pb 1.

The data also indicate the developmental expression of PUFA in four genotypes. Linoleic acid content of Shilajeet decreased continuously till 40 daf stage while little changes were

**Table 1. Lipoxygenase isozymes, polyunsaturated fatty acids and trypsin inhibitor content (db) during developmental stages in selected soybean varieties**

| Variety | Days after flowering | Lipoxygenases (Units/g) | | | Polyunsaturated fatty acids (%) | | Trypsin inhibitor (mg/g) |
|---|---|---|---|---|---|---|---|
| | | Lox I | Lox II+III | Total Lox | C 18:2 | C 18:3 | |
| JS 335 | 30 | 364 ± 9.4 | 142 ± 2.6 | 506 | 49.0±1.6 | 15.0 ± 0.86 | 48.0 ± 4.3 |
| | 35 | 532 ± 10.4 | 220 ± 13.7 | 752 | 46.8±1.67 | 9.0± 0.38 | 40.0 ± 4 |
| | 40 | 860 ± 6.8 | 380 ± 7.6 | 1240 | 48.0±1.0 | 7.9 ± 0.16 | 40.5 ± 4.5 |
| | 45 | 1380 ± 20 | 240 ± 6.5 | 1620 | 49.6±1.1 | 7.5± 0.22 | 39.5 ± 3.2 |
| | 50 | 1440 ± 23 | 290 ± 8.5 | 1730 | 53.4±2.2 | 7.0 ± 0.66 | 55.9 ± 1.9 |
| | 55 | 1358 ± 26 | 226 ± 12.6 | 1584 | 42.0 ±0.55 | 5.6 ± 0.46 | 47.0 ± 4.8 |
| | Maturity | 1360 ± 23 | 239 ± 14 | 1595 | 41.7±0.33 | 5.5± 0.32 | 52.0 ± 3.7 |
| Pb 1 | 30 | 118 ± 11 | 176 ± 12.3 | 294 | 42.7±0.67 | 12.2 ± 0.83 | 7.4 ± 0.6 |
| | 35 | 280 ± 1.3 | 185 ± 13.5 | 470 | 41.4 ± 1.2 | 9.8 ± .067 | 28.3 ± 1.7 |
| | 40 | 328 ± 9 | 198± 12.6 | 526 | 41.6 ± 1.3 | 7.5 ± 0.55 | 40.0 ± 1.2 |
| | 45 | 550 ± 12 | 212± 13.4 | 762 | 45.9± 0.76 | 7.0 ± 0.37 | 48.0 ± 3.2 |
| | 50 | 564± 14 | 254± 12.6 | 818 | 51.2 ± 1.8 | 7.2 ± 0.28 | 51.3 ± 3.1 |
| | Maturity | 624 ± 26 | 210 ± 16 | 834 | 51.4± 0.97 | 7.3 ± 0.66 | 65.0 ± 3.4 |
| NRC 37 | 30 | 68 ± 3.6 | 47 ± 2.4 | 115 | 46.9±1.74 | 15.9 ± 0.79 | 15.7 ± 1.1 |
| | 35 | 64± 6.5 | 130± 3.2 | 194 | 46.5±1.38 | 13.9 ± 0.89 | 27.4 ± 2.2 |
| | 40 | 460 ± 14.6 | 150 ± 6.5 | 610 | 46.4±1.55 | 11.0 ± .56 | 18.2 ± 1.3 |
| | 45 | 880 ± 12.4 | 230 ± 7.6 | 1110 | 50.7± 0.65 | 9.3 ± 0.55 | 35.6 ± 1.4 |
| | 50 | 1080 ± 13.6 | 395 ± 13.6 | 1475 | 52.0 ± 0.71 | 8.2 ± 0.33 | 33.6 ± 0.7 |
| | Maturity | 1264± 44 | 500 ± 15.4 | 1764 | 49.9 ± 0.66 | 7.9 ± 0.42 | 73.2 ± 3.1 |
| Shilajeet | 30 | 384± 12.2 | 160± 5.6 | 540 | 41.3 ±1.54 | 13.7± 0.53 | 10.3 ± 0.7 |
| | 35 | 410 ± 7.6 | 198± 13.2 | 608 | 36.2± 0.96 | 6.6 ± 0.77 | 20.7 ± 1.3 |
| | 40 | 520 ± 11.2 | 255± 12.6 | 775 | 36.8 ± 1.4 | 6.0 ± 0.54 | 30.4 ± 0.8 |
| | 45 | 736 + 6.5 | 280± 6.9 | 1016 | 38.0 ± 0.99 | 5.6 ± 0.65 | 50.0 ± 1.4 |
| | 50 | 984± 12.5 | 320 ± 3.6 | 1304 | 41.4 ± 1.5 | 5.2 ± 0.65 | 50.1 ± 2.1 |
| | 55 | 789 ± 36 | 466 ± 23 | 1255 | 42.0 ± 1.66 | 5.0 ± 0.43 | 71.6 ± 3.6 |
| | Maturity | 696 ± 18 | 248± 16 | 944 | 42.2 ± 1.22 | 5.0 ± 0.12 | 59.0 ± 2.3 |

*Values given are mean of triplicate samples ± standard deviation*

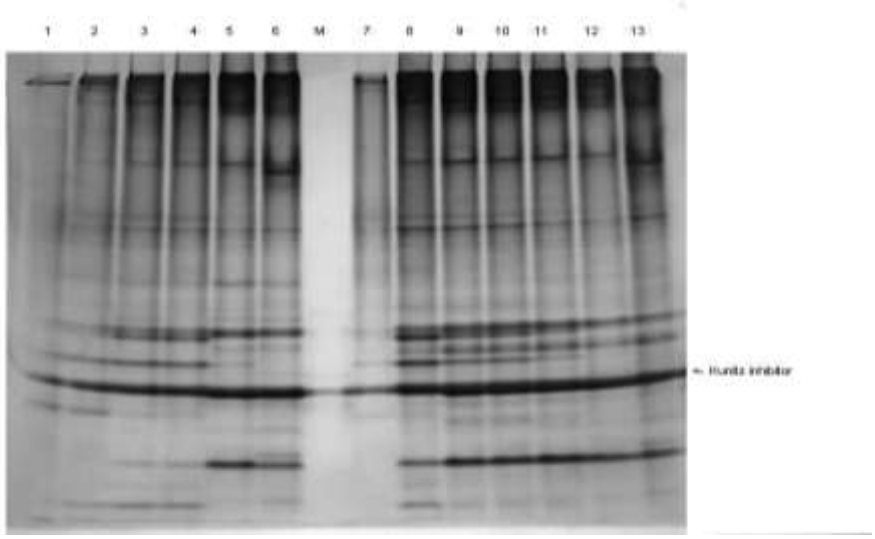


**Fig 1. Polyacrylamide gel electrophoresis of NRC 37 and JS 335 during seed development. Lanes 1-6 represent 30, 35, 40, 45, 50 daf and at harvest maturity of NRC 37, while lanes 7-13 represent 30, 35, 40, 45, 50, 55 daf and at harvest maturity, respectively in JS 335. Lane M represents the marker protein for Kunitz inhibitor**

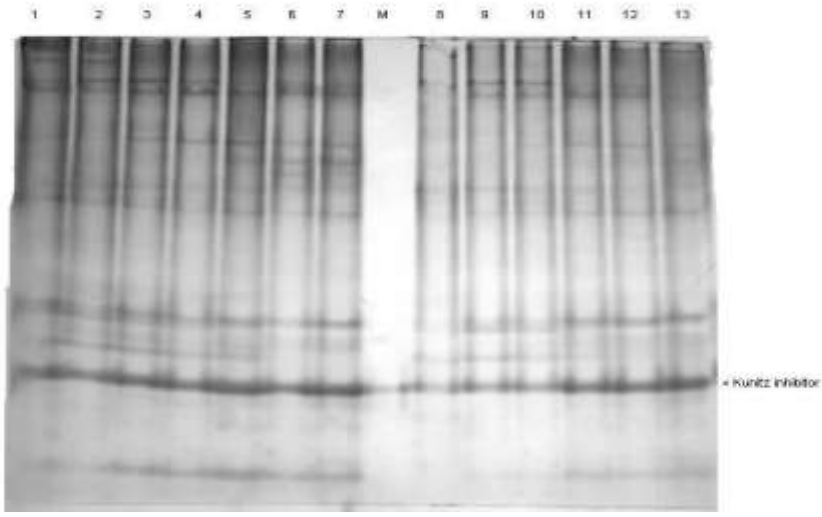


**Fig 2. Polyacrylamide gel electrophoresis of Shilajeet and Pb 1 during seed development. Lanes 1-7 represent 30, 35, 40, 45, 50 daf and at harvest maturity of Shilajeet, while lanes 8-13 represent 30, 35,40, 45, 50 daf and at harvest maturity, respectively of Pb 1. Lane M represents the marker protein for Kunitz inhibitor**

**Table 2. Correlation studied among lipoxygenase isozymes, trypsin inhibitor and polyunsaturated fatty acids of a few selected soybean varieties during seed development**

| | Lox II+III | Total Lox | Linoleic acid C 18:2 | Linolenic acid C 18:3 | Trypsin inhibitor |
|---|---|---|---|---|---|
| Lox 1 | NS | .987*** | NS | -0.620** | 0.579** |
| Lox II+III | | NS | NS | -0.381* | NS |
| Total Lox | | | | -.658*** | .631** |
| Linoleic acid | | | | NS | NS |
| Linolenic acid | | | | | -0.620** |

**, **, *** indicate significance at p<0.05, p<0.01 and p<0.001 respectively*

observed in NRC 37, JS 335 and Pb 1. Furthermore, as the maturity approached, JS 335 exhibited decline for linoleic acid, while in Pb 1 and Shilajeet it kept on increasing till maturity.

Linolenic acid content was found to be maximum at 30 daf and thereafter decreased continuously in all the genotypes till maturity though at varying rate. Maximum drop of 9.5 percent in linolenic acid content was observed in JS 335 followed by Shilajeet as the seed development stage advanced from 30 daf to maturity. The continuous decline of linolenic acid during seed development is in contrast to findings of Sangwan *et al.* (1986) reported that an increase in the level of linolenic acid during seed development but observations in the present study are in consonance with other reports (Dornbos and McDonald 1986, Rubel *et al.* 1972). It was interesting to observe that the maximum decrease in linolenic acid content of all the five genotypes was between the development stage of 30 daf and 35 daf. Furthermore, in the very early stage of development when linolenic acid is very high, lipoxygenase isozymes levels are low. The correlations between different biological components during seed development are given in Table 2. TI showed significant correlations with total lipoxygenase and lipoxygenase-I ($p<0.01$). Linolenic acid showed significant negative correlation with total lipoxygenase ($p<0.001$), lipoxygeanse I ($p<0.01$) and lipoxygenase-II+III ($p<0.01$). Significant negative correlation was observed between TI and linolenic acid ($p<0.01$).

Significant positive correlation of TI with lipoxygenase-I observed in the present study suggests coordinated expression of two undesirable components of soybean during seed development. Lipoxygenase present in the leaves of several plants species have been implicated in the defense against pathogens and insects (Siedow 1991). In tomato leaves, it has been proposed that linolenic acid hydroperoxides derived from lipoxygenases activity are precursor of jasmonic acid which would in turn activate transcription of genes encoding for protease inhibitors which have a role against insect attack (Farmer and Ryan 1992). In soybean leaves, members of cysteine proteinase inhibitor are regulated by jasmonate. Furthermore, presence of jasmonic acid as detected in developing soybean seeds (Lopez *et al.* 1987) and reduced level of proteinase inhibitor in null lipoxygenase lines as reported by Carvalho *et al.* (1999) indicate similar physiological process as observed in the present study. However, in developing seeds whether jasmonates resulted from lipoxygenases' derived hydroperoxides activate the synthesis of protease inhibitor need to be investigated.

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# Exploiting the Potential of *Bacillus thuringiensis* in the Management of Lepidopterous Defoliators Infesting Soybean

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**ABSTRACT**

*Soybean crop suffers substantial yield losses on account of insect-pest infestation. To reduce these losses only chemical insecticides are relied upon. But due to their indiscriminate use, several problems are gradually cropping up. Microbial insecticides, particularly Bacillus thuringiensis based, have shown great potential in the management of soybean insect-pests. Results of research work conducted on various aspects viz. efficacy, integration and compatibility with chemical insecticides, economic feasibility, field demonstrations etc. are presented in this paper. Besides, certain limitations encountered with large-scale adoption of microbial insecticides are also discussed.*

**Key words:** *Bacillus thuringiensis*, defoliators, management, compatibility, microbial insecticide

Soybean [*Glycine max* (L) Merrill] in India is attacked by about 20 major insect pests, of which lions share is taken by a dozen of lepidopterous defoliators. Uncontrolled insect pest complex is responsible for yield reduction to the tune of 27 per cent (Sharma and Shukla 1997). Chemical insecticides recommended for their control do reduce their population. But due to the injudicious use of chemical insecticides, the inadvertent problems are emerging very fast (Mehrotra 1991). Bio-pesticides, especially insect pathogen based, have exhibited great potential in the management of major insect pests of soybean (Sharma and Ansari 1999; Dutta and Sharma 1997). Among such bio-pesticides, those containing *Bacillus thuringiensis* (Bt) have been widely tested against insect-pests of soybean. Owing to their comparable efficacy and eco-friendly nature, they tend to become an integral component of viable Integrated Pest Management (IPM) programme. Bt also offers great promise for the management of insects that have developed resistance against chemical insecticides. Present paper gives an account of research work carried out on different aspects of Bt for the management of soybean insect-pests, especially lepidopterous defoliators.

## Efficacy of Bt against soybean insect-pests

Efficacy of five commercially available Bt formulations *viz.* Delfin, Biolep, Dipel, Bioasp and Biobit, was tested against two major

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defoliators *viz*. *Spodoptera litura* and *Spilarctia obliqua* under controlled conditions. Exposure to Biobit, Bioasp and Dipel resulted in conspicuous larval mortality in 4-5 days (Sharma 2000). Studies conducted with these Bt based microbial insecticides and entomo-pathogenic fungus, *Beauveria bassiana*, under natural field conditions also showed similar results, where the larval population started declining in 3 days after treatment (DAT) (Fig. 1). After 10 days after treatment (DAT), the mortality with Bt was comparable with that of triazophos (Fig. 2).

As the cost of commercial Bt formulations is very high in comparison to chemical insecticides, a low cost formulation was prepared from Bt isolated from field infected *S. litura* larvae. This formulation also gave reasonably good control of lepidopterous defoliators (Fig. 3). The procedure of preparing the formulation is briefly described below.

***Isolation of Bt:*** Few *S. litura* larvae having bacterial infection were collected from the field. After surface sterilization they were crushed individually in sterile distilled water. The exudates were streaked aseptically with a bent glass rod on nutrient agar medium already poured in a sterile Petridish. The plates were incubated at $25^0$ C for 72-96 hours. The individually isolated Bt colonies were picked up on nutrient agar slant for further studies.

***Pathogenicity:*** Forty-eight hours old bacterial culture was washed in sterile distilled water and the concentration was adjusted to $10^8$ CFU/ml. The bacterial suspension was sprayed on soybean leaves, which were dried in shade and fed to first and second instar *Spodoptera* larvae in laboratory condition. The dead larvae were dissected and the Bt association was confirmed. The isolation of these larvae again yielded the same Bt colonies.

***Preparation of Bt formulation for field test:*** Twenty grams soybean seeds were soaked in water for overnight, crushed in a pestle mortar and then boiled in 1 liter of water for 5 minutes. The suspension was filtered through three layers of cheese cloth and the final volume was again adjusted to 1 liter, and poured in 250 ml Erlenmeyer flasks and sterilized for 20 minutes under 15 pounds pressure. The soybean broth was then inoculated aseptically with 1 ml of 48-72 hours old bacterial culture and incubated at room temperature for 4-6 days. After incubation the bacterial culture was further diluted to 1:4 (v/v) and used for field spraying.

**Integration of Bt with chemical insecticides**

Usually soybean is sprayed twice with chemical insecticides to control insect-pests. But some affluent farmers do not hesitate to go for 3 or even 4 sprays without assessing the need, thereby increasing the unwarranted load of chemical insecticides as well as cost of cultivation. Trials were conducted to rationalize the use of chemical insecticides and to assess the possibility of incorporating Bt in spray schedule. Results showed that whether applied alone or integrated in spray schedule of chemical insecticide, Bt formulations suppressed the population of lepidopterous defoliators significantly (Table 1). Results also indicated that if integrated with Bt, it was possible to reduce the dose of chemical insecticide to half of the recommended dose without compromising the efficacy (Fig. 4).

**Demonstration at Farmers' fields**

As stated earlier, Bt based microbial insecticides form an important component of IPM, field demonstrations were conducted under real farm conditions at farmers' fields during 1997 and 1998. The IPM package included following components.

- Deep summer ploughing
- Use of insect tolerant variety
- Application of phorate @ 10 kg/ha at sowing

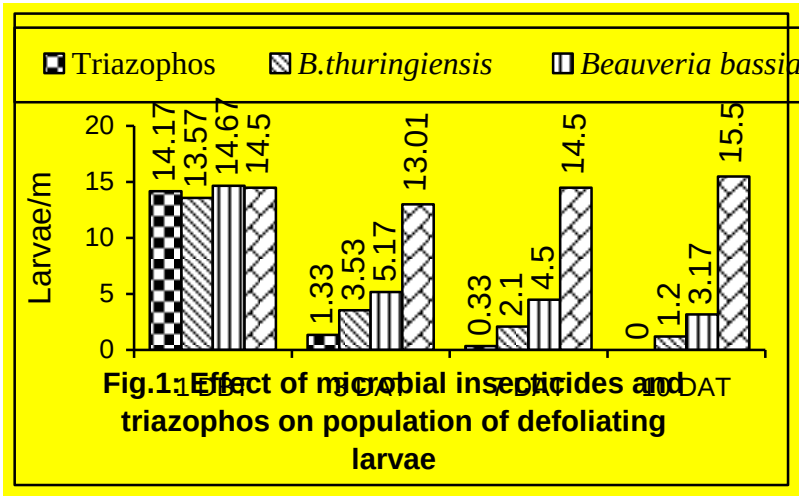


**Fig.1: Effect of microbial insecticides and triazophos on population of defoliating larvae**

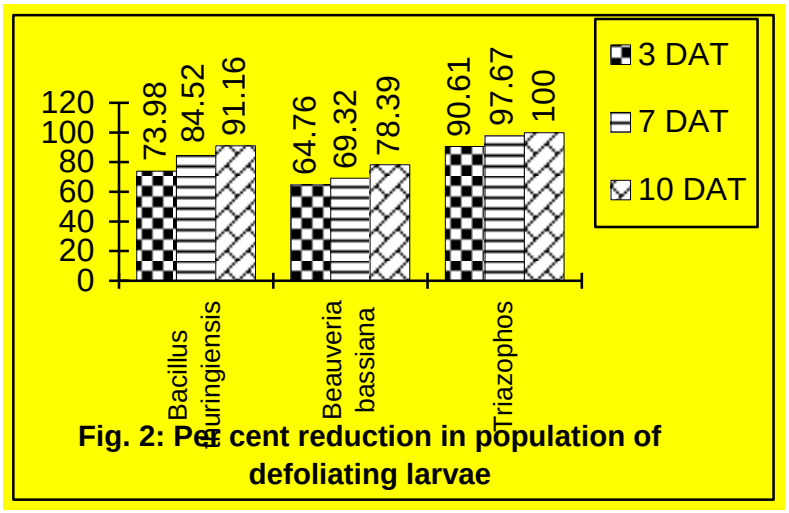


**Fig. 2: Per cent reduction in population of defoliating larvae**

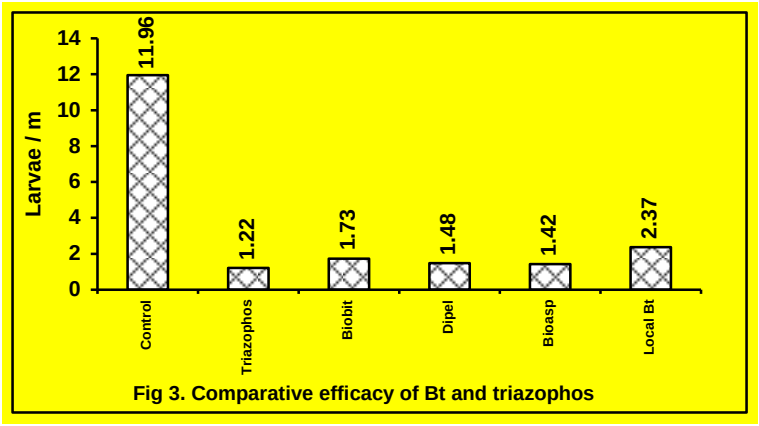


**Fig 3. Comparative efficacy of Bt and triazophos**

**Table 1. Effect of Bt formulations and chemical insecticide on population of lepidopterous defoliators**

| Treatment | Larval population per m row | | | | | Grain yield (kg/ha) |
|---|---|---|---|---|---|---|
| | 1 DBT | 3 DAT | 7 DAT | 10 DAT | Mean | |
| Triazophos | 9.40 (3.14)* | 2.50 (1.73) | 0.97 (1.21) | 0.20 (0.83) | 1.22 (1.30) | 1847 |
| Biobit | 8.73 (3.04) | 3.60 (2.02) | 1.17 (1.28) | 0.43 (0.96) | 1.73 (1.49) | 1798 |
| Biobit + Triazophos | 8.83 (3.05) | 2.33 (1.68) | 0.57 (1.03) | 0.20 (0.83) | 1.03 (1.23) | 1884 |
| Biobit + ½ Triazophos | 8.67 (3.03) | 2.87 (1.83) | 1.03 (1.24) | 0.43 (0.96) | 1.44 (1.39) | 1874 |
| Dipel | 9.17 (3.11) | 3.17 (1.91) | 0.93 (1.19) | 0.37 (0.92) | 1.48 (1.41) | 1781 |
| Dipel + Triazophos | 9.13 (3.10) | 2.03 (1.59) | 0.50 (0.99) | 0.10 (0.77) | 0.87 (1.17) | 1949 |
| Dipel + ½ Triazophos | 8.80 (3.05) | 2.67 (1.78) | 0.73 (1.10) | 0.40 (0.94) | 1.26 (1.33) | 1863 |
| Bioasp | 9.47 (3.16) | 3.00 (1.87) | 1.00 (1.21) | 0.27 (0.87) | 1.42 (1.38) | 1750 |
| Bioasp + Triazophos | 9.40 (3.15) | 1.47 (1.40) | 0.37 (0.92) | 0.20 (0.83) | 0.64 (1.06) | 1920 |
| Bioasp + ½ Triazophos | 8.87 (3.06) | 2.03 (1.59) | 0.70 (1.09) | 0.20 (0.83) | 0.97 (1.21) | 1897 |
| Local Bt | 9.33 (3.13) | 4.36 (2.26) | 1.83 (1.53) | 0.70 (1.09) | 2.37 (1.69) | 1760 |
| Control | 9.80 (3.21) | 10.70 (3.34) | 11.86 (3.52) | 13.33 (3.72) | 11.96 (3.53) | 1512 |
| SEm (±) | -- | (0.05) | (0.08) | (0.07) | (0.04) | 44 |
| **LSD (P=0.05)** | **(NS)** | **(0.15)** | **(0.24)** | **(0.20)** | **(0.13)** | |

***Dose**: All Bt formulations - @ 1 kg/ha, Triazophos 40EC - @ 0.8 l/ha, 1/2 Triazophos - @ 0.4 l/ha, Local Bt - $10^8$ CFU/ml; * Square Root transformed values are given in parentheses.*

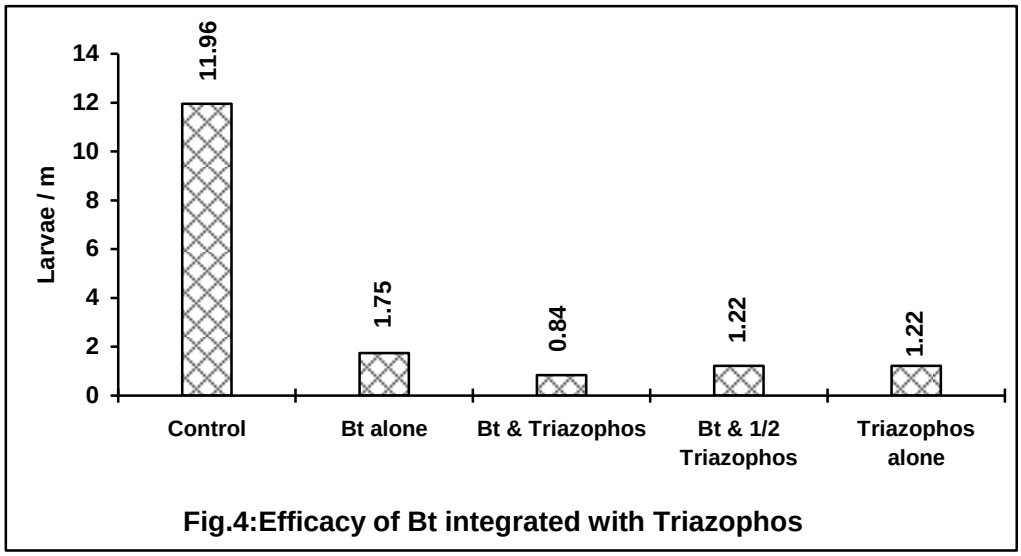


**Fig.4:Efficacy of Bt integrated with Triazophos**

- Installation of light-trap
- Use of bird perches

- Manual removal of plant parts infested with girdle beetle and *S. litura*
- Spray of Bt based bio-pesticide (Dipel @ 1.0 lit/ha)
- Need based application of chemical insecticide (triazophos 40 EC @ 0 .8 lit/ha)

Although the yields realized during year 1998 were low due to bad weather conditions, still the average yield obtained through IPM was 397 kg/ha more that that obtained by farmers' practice (Table 2). Despite the fact that costly input like phorate and Bt formulation (Dipel) were used in IPM, the economic returns to the farmers were substantial. The immediate monitory benefit to the farmers was to the tune of Rs. 2273/- per ha (Table 3). Although the farmers were convinced with the use of Bt and IPM technology *per se*, they did not sustained their use owing to high cost involved.

**Compatibility of Bt with chemical insecticides and fungicides**

Typically, soybean is attacked almost simultaneously by defoliating larvae as well as stem borers (stem fly and girdle beetle). Under such conditions it becomes operationally inconvenient to apply different kinds of insecticides (contact or systemic) at one time. Usual practice adopted by the farmers is to apply contact insecticide first followed by one or two sprays of systemic insecticides. Situation becomes unmanageable when soybean gets infected with foliar diseases also. In order to cope up with such complex situations, attempts were made to test the efficacy of tank-mix formulations of Bt, chemical insecticide and / or fungicides. As a prerequisite, compatibility of Bt with chemical insecticide and fungicides recommended for soybean was assessed under laboratory conditions. In this study, Bt was found to be compatible with monocrotophos, carbendazim, thiophenate methyl and triademefon (Table 4). Further, Bt was also found compatible with mixture of monocrotophos + carbendazim and monocrotophos + thiophenate methyl (Ansari and Sharma 2000 a, b). Pramanik *et al.* (1997) also found that monocrotophos did not inhibit the growth of Bt while quinalphos and endosulfan were toxic and inhibited the growth of Bt.

Efficacy of these compatible combinations was also tested under natural field conditions (Table 5). The results explicitly confirmed that it was possible to contain population of defoliators and damage due to stem fly and girdle beetle through single spray of above-mentioned compatible combinations as tank-mix formulations. In soybean rust prone areas, mixture of Bt + triademefon can be sprayed to control defoliators and soybean rust. The yield advantage with Bt and its compatible combinations range from 18 to 32 percent over control, maximum being with spray of Bt + monocrotophos + carbendazim. The increase in the efficacy of biological component (Bt) of the mixture in tank mix formulation of chemical and bio-pesticide was also reported by Jaques (1988).

In another laboratory experiment with a set of newly recommended insecticides, Bt was found to be compatible with Lufenuron 5 EC (@ 0.4 lit/ha), Thiamethoxam 25 G (@ 100 g/ha) and Methomyl 40 SP (@ 1.0 kg/ha); while Ethion 50 EC (@ 1.5 l/ha) completely inhibited the growth of Bt colonies (Table 6).

**Economic feasibility**

Success of any technology undoubtedly lies in its economic feasibility. Although, potential of Bt has been well acclaimed with respect to its efficacy against insect-pests and yield advantage, yet the extent of adoption is meager. In Indian context, poor adoption of Bt is attributed mainly to higher cost of commercial formulations that are manufactured using imported technical material (strains). Although, when used as

**Table 2. Soybean yield realized through IPM technology and Farmers' practice**

| Location | IPM technology | | | Farmers' practice | | |
|---|---|---|---|---|---|---|
| **No.** | **1997** | **1998** | **Mean** | **1997** | **1998** | **Mean** |
| 1. | 2089 | 1000 | 1545 | 1544 | 350 | 947 |
| 2. | 1109 | 1152 | 1131 | 1130 | 460 | 795 |
| 3. | 2423 | 1012 | 1718 | 2400 | 380 | 1390 |
| 4. | 2462 | 1000 | 1731 | 750 | 500 | 625 |
| 5. | 2037 | 560 | 1299 | 1200 | 340 | 770 |
| 6. | 1450 | 670 | 1060 | 1130 | 650 | 890 |
| 7. | 1896 | 1020 | 1458 | 1730 | 890 | 1190 |
| 8. | 2324 | 967 | 1650 | 1880 | 1260 | 1570 |
| 9. | 2085 | 1008 | 1547 | 1500 | -- | 1500 |
| 10. | 1975 | 1262 | 1619 | 1400 | -- | 1400 |
| 11. | -- | 1800 | 1800 | -- | -- | -- |
| **Mean** | **1985** | **1041** | **1505** | **1460** | **604** | **1108** |

**Table 3. Monitory benefits through IPM technology**

| | **IPM technology** | **Farmers' practice** |
|---|---|---|
| Total produce | 1505 kg/ha | 1108 kg/ha |
| Value of produce | Rs. 13545 / ha | Rs. 9972 / ha |
| Expenditure on insect control | Rs. 1900 / ha | Rs. 600 / ha |
| Net profit | Rs. 11645 / ha | Rs. 9372 / ha |

a component of IPM, Bt promises significant economic advantage, yet when compared with chemical insecticides, its economic feasibility lags behind. In two years field trials, application of Bt recorded an average additional yield of 419 kg/ha that was comparable with best treatment - quinalphos (467 kg/ha). But, on account of higher cost of Bt, the cost: benefit ratio (CBR) was very less (7.33) as compared to chemical insecticides (Table 7). Nevertheless, it is expected that the low economic returns with Bt will not be a limiting factor, once its long-term benefits are realised and taken into account.

**Limitations**

***Lack of awareness***: A large proportion of farmers is ignorant of use of Bt in insect-pest management and still relies on chemical insecticides. Needed attention is lacking both with respect to acquiring technical knowledge and transmitting to the farmers, in the extension functionaries.

***Inadequate production of Bt based insecticides:*** Production and supply of Bt based insecticides in major soybean growing area is very meager because of poor market development efforts by the manufacturers. No government subsidy is available on these products.

***Easy access to chemical insecticides:*** Owing to quick knock down and visual effect, affordable prices and strong market, chemical insecticides dominate insect management practices. Farmers mostly depend on non-technical

**Table 4. Bacterial colonies in the media fortified with insecticides or fungicides**

| Treatment | No. of CFU / ml |
|---|---|
| Control (Bt alone) | $6.36 \times 10^9$ |
| Bt + monocrotophos | $5.92 \times 10^9$ |
| Bt + endosulfan | Nil |
| Bt + triazophos | Nil |
| Bt + quinalphos | Nil |
| Bt + carbendazim | $5.42 \times 10^9$ |
| Bt + thiophanate methyl | $5.54 \times 10^9$ |
| Bt + triademefon | $4.93 \times 10^9$ |
| Bt + mancozeb | Nil |
| Bt + chlorothalonil | Nil |
| Bt + hexaconazol | Nil |
| Bt + copper oxychloride | Nil |
| Bt + diafenconazol | Nil |
| Bt + propiconazol | Nil |
| Bt + tridemorph | Nil |
| Bt + monocrotophos + carbendazim | $5.60 \times 10^9$ |
| Bt + monocrotophos + thiophanate methyl | $5.70 \times 10^9$ |
| Bt + monocrotophos + triademefon | Nil |

**Table 5: Effect of Bt and compatible pesticides on insect population / damage and grain yield (Pooled data of three years)**

| Treatment / Dose | Blue beetle / m | Semiloopers / m | Stem tunnelling (%) | Girdle beetle damage (%) | Grain yield (kg/ha) |
|---|---|---|---|---|---|
| *Bacillus thuringiensis* @ 1.0 l/ha | 3.55 (43.91) * | 4.30 (63.74) * | 10.28 (63.01) * | 28.20 (56.04) * | 2581 (22.21) # |
| Monocrotophos @ 0.8 l/ha | 1.67 (73.62) | 3.25 (72.60) | 11.18 (59.77) | 24.54 (61.74) | 2631 (24.46) |
| T-1 + T-2 | 2.44 (61.45) | 2.25 (81.03) | 9.93 (64.27) | 22.20 (65.39) | 2586 (22.44) |
| T-1 + Carbendazim @ 0.05 % | 3.00 (52.61) | 4.28 (63.91) | 10.99 (60.45) | 33.18 (48.28) | 2545 (20.50) |
| T-1 + Thiophenate Methyl (Topsin M 70 WP) @ 0.05 % | 3.11 (50.87) | 4.17 (64.84) | 12.00 (56.82) | 42.57 (33.64) | 2501 (18.42) |
| T-1 + Triademefon @ 0.05 % | 3.00 (52.61) | 3.94 (66.78) | 14.09 (49.30) | 27.94 (56.44) | 2555 (20.97) |
| T-3 + Carbendazim | 1.33 (78.99) | 2.41 (79.68) | 10.71 (61.46) | 27.25 (57.52) | 2790 (32.10) |
| T-3 + Thiophenate Methyl | 1.67 (73.62) | 2.08 (82.46) | 9.24 (66.64) | 21.17 (67.00) | 2734 (29.45) |
| Control | 6.33 (--) | 11.86 (--) | 27.79 (--) | 64.15 (--) | 2112 ( -- ) |

** Per cent reduction in insect population / damage given in parentheses; # Per cent additional yield given in parentheses*

**Table 6. *In vitro* compatibility of *Bacillus thuringiensis* with chemical insecticides**

| Treatment / Dose | Bt* (CFU/ml) |
|---|---|
| Control | $13.77 \times 10^4$ |
| Lufenuron 5EC (Match) @ 0.4 lit/ha | $13.20 \times 10^4$ |
| Thiamethoxam 25WG (Actra) @ 300 g/ha | $12.92 \times 10^4$ |
| Methomyl 40SP (Lannate) @ 1.0 kg/ha | $13.93 \times 10^4$ |
| Ethion 50EC (Fosmite) @ 1.5 lit/ha | 0 |

** Average of 6 replications*

retailers for advice. Often, the farmers incur economic loss by using sub standard insecticides.

***Farmers' economic status:*** As the microbial insecticides are manufactured by the private sector with imported strains the production cost increases and the product becomes beyond the financial reach of a common farmer. In retail market microbial insecticides are at least 2 to 3 times costlier than chemical insecticides.

***Lack of incentives:*** There is no financial incentive either from government or from industry for soybean produced without using chemical insecticides. Even the minimum support price (MSP) does not account for the actual cost of cultivation using costly microbial insecticides.

**Areas that need attention**

The information contained in this article amply signifies of the potential role Bt can play in the management of soybean insect-pests, especially the defoliators. However, there is dire need to address certain issues either through concerted research efforts or through policy intervention. Areas that need attention of scientists, industry and / or policy makers are briefly presented below.

- Identification, testing and commercialization of native strains to bring down the production cost (Action: Scientists and Industry).
- Genetic manipulation to improve the efficacy of native strains (Action: Biotechnologists)
- Stringent quality control of existing commercial formulations (Action: Government and CIB)
- Mass awareness programmes through Field Demonstrations and Trainings to farmers as well as extension personnel (Action: Government, Research Organizations and NGOs)
- Promotion of cottage level industry for production of microbial insecticides (Action: Government and NGOs)
- Monitoring for development of resistance against Bt (Action: Entomologists)
- Special pricing (Minimum Support Price) for soybean produced using biological (microbial) insecticides (Action: Central Government, Industry and State Mandi Boards)

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**Table 7. Economics of various insecticides recommended for use in soybean (Pooled data of 2002 and 2003)**

| Treatment / Dose | Yield (kg/ha) | | | | Price of increased yield (Rs.) | Cost of treatment (Rs.) | Incremental CBR |
|---|---|---|---|---|---|---|---|
| | 2002 | 2003 | Mean | Increase over control | | | |
| Chlorpyrifos 20 EC @ 1.5 l/ha | 2033 | 2846 | 2440 | 441 | 6174 | 255 | 24.21 |
| Triazophos 40 EC @ 0.8 l/ha | 2046 | 2857 | 2452 | 453 | 6342 | 340 | 18.65 |
| Quinalphos 25 EC @ 1.5 l/ha | 2013 | 2918 | 2466 | 467 | 6538 | 375 | 17.43 |
| Methomyl 40 SP @ 1.0 kg/ha | 1790 | 2854 | 2322 | 323 | 4522 | 1150 | 3.93 |
| Ethion 50 EC @ 1.5 l/ha | 1833 | 2856 | 2345 | 346 | 4844 | 375 | 12.92 |
| Ethofenprox 10 EC @ 1.0 l/ha | 1740 | 2768 | 2254 | 255 | 3570 | 700 | 5.10 |
| Thiamethoxam 25 WG @ 100 g/ha | 1815 | 2963 | 2376 | 377 | 5278 | 425 | 12.42 |
| Endosulfan 35 EC @1.5 l/ha | 1802 | 3024 | 2413 | 414 | 5796 | 322 | 18.00 |
| Monocrotophos 36 SL @ 0.8 l/ha | 1904 | 2798 | 2352 | 353 | 4942 | 220 | 22.46 |
| Dipel 8 L @ 1.0 l/ha | 2009 | 2827 | 2418 | 419 | 5866 | 800 | 7.33 |
| Control | 1503 | 2496 | 1999 | -- | -- | -- | -- |

*Price of Soybean = Rs. 14.00 / kg; Chlorpyrifos = Rs. 170.00 / l ; Triazophos = Rs. 425.00 / l ; Quinalphos = Rs. 250.00 / l ; Methomyl = Rs. 1150.00 / kg ; Ethion = Rs. 250.00 / l ; Ethofenprox = Rs. 700.00 / l ; Thiamethoxam = Rs. 4250.00 / kg ; Endosulfan = Rs. 215 / l ; Monocrotophos = Rs. 275.00 / l ; Dipel (Bt) = Rs. 800.00 / l*


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# Effect of Doses and Sources of Sulphur on Nodulation, Yield, Oil and Protein Content of Soybean and Soil Properties

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**ABSTRACT**

*An experiment for evaluating the effect of different doses and sources of fertilizer sulphur in soybean cv. JS 335 (*Glycine max *L. Merrill) was conducted under on a Typic Vertisol (Sarol series) in randomized block design with 4 replications at Research Farm, College of Agriculture, Indore. Incorporation of N and P in the nutrition of soybean increases the yield marginally (8%) but with incorporation of S the yields increased by 45-49%, 68-69% and 80 – 87% with 10, 20 and 30 kg S ha$^{-1}$, respectively. Complex fertilizer (13-33-0-15S) was superior to mixed source i.e. ½ dose as ammonium sulphate + ½ as elemental sulphur. The results revealed that the application rate of sulphur should be elevated from 15/ 20 kg/ ha S to 30 kg S/ ha. The fertilizer (13-33-0-15S) was found to be significantly superior in increasing the nodulation, yield attributes, uptake of phosphorus, potassium, and DTPA zinc, oil and protein content in soybean.*

**Key words:** Sources of sulphur, complex fertilizer, nodulation, nutrient uptake, soil properties

Madhya Pradesh is known as "Soybean State" with acreage of 4.44 million hectares (covering nearly 74% of total area under soybean in India) producing 4.74 million tons (70%) of soybean (Agricultural Statistics 2001). Soybean occupies about 67 percent of the area in Madhya Pradesh, of which nearly 47 percent lies in agro-climatic zone X (*Malwa* plateau) and 20 percent in agro-climatic zone V (*Vindhyan* plateau) having vertisols and associated soils exhibiting clay texture and high water holding capacity.

About 50 percent soils of the *Malwa* plateau have been reported to be deficient in S (Tomar *et al.,* 1995). With increasing cropping intensity and use of complex fertilizers, deficiency of sulphur has become a common nutritional problem. The application of sulphur @ 15 –20 kg S/ha has been recommended in package of practices of soybean, which is generally being supplied through gypsum (only 0.2% solubility). Another source of supply of sulphur is through agricultural grade pyrites, which is to be applied on the soil surface, which has field capacity moisture regime for 7 days thereby involving complex phenomenon. The depletion of sulphur is occurring to a greater tune than the addition thereby calling for higher rate of application. The vagary of the farmer is non-availability of any single fertilizer, which can provide balance nutrition of N, P and S to soybean in prilled form, which can be drilled.

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A new complex fertilizer carrying 13-33-0-15S as N:P:K:S is being developed by M/S Cargill India Pvt. Ltd. is in prilled form and may provide a good fertilizer substitute for soybean growers as it can be drilled simultaneously with seeding. Thus, the experiment to asses the effect of doses and sources of sulphur on nodulation, oil and protein content and nutritional value of soybean was undertaken in vertisols of *Malwa* plateau of Madhya Pradesh.

## MATERIAL AND METHODS

An experiment for evaluating the effect of different doses and sources of fertilizer sulphur on soybean (*Glycine max* L. Merrill) cv. JS 335 was conducted on a bench mark *Sarol* soil series (Murthy *et al*., 1981) belonging to fine montmorillonitic hyperthermic family of Typic Haplusterts at Experimental Farm, College of Agriculture, Indore in randomized block design with 8 treatments replicated 4 times for two consecutive years (2002-03). Sulphur was applied in 4 doses @ 0, 10, 20 and 30 kg S/ ha and was supplied through ½ through ammonium sulphate (AS) + ½ as elemental sulphur (ES) and complex fertilizer (13-33-0-15S) provided by M/S Cargill India Pvt. Limited, Gurgaon with recommended NPK fertilizers and one absolute control. N: $P_2O_5$: $K_2O$ were applied uniformly @ 31: 66: 33 kg/ ha in all the treatments. The mode of application was basal. The plot size maintained for each treatment was 50 $m^2$. The climate of the area is semi-arid subtropical monsoon type with an average annual rainfall of 979 mm, most of which is received between June and September. However, during the experimental period the precipitation was only 675.6 mm (69% of the annual mean rainfall).

The initial analysis revealed that the soils are alkaline in reaction (pH- 8.0), are non-saline non-alkali (EC– 0.33 dS/m) and clay in texture with high CEC (50.50 cmol(p+)/kg). It exhibited medium organic carbon (0.56%), available nitrogen (221.4 kg N/ ha), phosphorus (11.1 kg $P_2O_5$/ ha) and sulphur (15.8 kg S/ ha) and high available potassium (750.5 kg $K_2O$/ ha). The DTPA extractable zinc (2.24 mg Zn/ kg) was above the critical limit (0.6 mg/ kg) for black soil.

Randomly selected 5 plants were collected at harvest for studying biometrical parameters viz. number of pods/ plant, number of seed/ pod and test weight. The seed and biomass was preserved and utilized for the analysis of N, P, K, S, Zn and protein and oil content. The seed and biomass yield were recorded plot-wise, pooled for two experimental years and analyzed statistically (Panse and Sukhatme 1954). The soil samples after harvest of soybean were collected from each plot and were analyzed as per standard methods for pH, EC (1:2), organic carbon, available nitrogen, phosphorus, potassium, sulphur and DTPA extractable zinc.

## RESULTS AND DISCUSSION

### Nodulation

The incorporation of sulphur in fertilization schedule resulted better symbiotic traits as compared to control. The nodule number, their fresh weight and dry weight per plant increased with increasing levels of sulphur from 10 to 30 kg/ha. Among sources, these traits were superior in case of sulphur application through complex fertilizer (13:33:0:15) than combined application of ammonium sulphate and elemental sulphur. Highest number of nodules (113.6), fresh weight (1.082 g) and dry weight (0.341 g) of nodules per plant was recorded when S is applied in the form of 13-33-0-15S @ 30 kg S/ha (Table 1), whereas minimum values were associated with absolute control (without N, P, K and S). These traits were superior to control in case of combined doses of nitrogen, phosphorus and potassium without sulphur.

**Table 1. Effect of different doses and sources of sulphur on number of nodules, fresh and dry weight nodule**

| Treatments | Nodules/ plant (No.) | Fresh weight (g) of nodules/ plant | Dry weight (g) of nodules/ plant |
|---|---|---|---|
| $NPKS_0$ | 83.0 | 0.836 | 0.252 |
| $NPKS_{10}$ (½AS+½ES) | 88.4 | 0.882 | 0.263 |
| $NPKS_{20}$ (½AS+½ES) | 100.6 | 0.917 | 0.278 |
| $NPKS_{30}$ (½AS+½ES) | 108.6 | 0.972 | 0.310 |
| $NPKS_{10}$ (13-33-0-15S) | 99.2 | 0.908 | 0.272 |
| $NPKS_{20}$ (13-33-0-15S) | 105.2 | 0.924 | 0.281 |
| $NPKS_{30}$ (13-33-0-15S) | 113.6 | 1.082 | 0.341 |
| $N_0P_0K_0S_0$ | 75.2 | 0.815 | 0.221 |

**Table 2. Effect of different doses and sources of sulphur on yield attributes, grain and straw yield of soybean**

| Treatments | Pods/ plant (No.) | Seeds/ pod (No.) | 100 grain weight (g) | Grain yield (kg/ ha) | Straw yield (kg/ ha) |
|---|---|---|---|---|---|
| $NPKS_0$ | 26.2 | 1.94 | 10.28 | 1007 | 1264 |
| $NPKS_{10}$ (½AS+½ES) | 27.9 | 1.95 | 10.92 | 1357 | 1703 |
| $NPKS_{20}$ (½AS+½ES) | 35.5 | 1.97 | 10.97 | 1570 | 2003 |
| $NPKS_{30}$ (½AS+½ES) | 41.3 | 2.00 | 11.21 | 1682 | 2145 |
| $NPKS_{10}$ (13-33-0-15S) | 31.0 | 1.98 | 11.06 | 1388 | 1717 |
| $NPKS_{20}$ (13-33-0-15S) | 38.6 | 2.04 | 11.53 | 1564 | 1936 |
| $NPKS_{30}$ (13-33-0-15S) | 50.0 | 2.08 | 11.94 | 1747 | 2214 |
| $N_0P_0K_0S_0$ | 21.0 | 1.92 | 10.19 | 931 | 1182 |
| SEm ± | 0.332 | 0.024 | 0.03 | 4.95 | 9.35 |
| **CD 5%** | **0.976** | **0.070** | **0.10** | **14.55** | **27.51** |

**Yield and yield parameters**

Significant increase in yield parameters viz. number of pods/ plant, number of seeds/pod, 100 grains weight, and seed and straw yield of soybean (Table 2) was noticed with the increasing doses and as well as sources of sulphur as compared to control. The maximum number of pods/plant (50.0), number of seeds per pod (2.08), 100 grains weight (11.94 g), seed yield (1747 kg/ha) and straw yield (2214 kg/ha) was recorded under $NPKS_{30}$ amended through complex fertilizer (13-33-0-15S), whereas minimum values were recorded under control. The combined influence of yield attributing characters appears to be instrumental for causing the differences in yield under different treatments. Similar were the observations of Fazal and Sisodia (1989) and Mishra and Agrawal (1994). Singh and Singh (2004) reported that grain and straw yield of black gram increases significantly with the increasing doses of S application.

Application of recommended NPK fertilizers could increase the soybean yield by 8 percent only over $N_0P_0K_0S_0$. The incorporation of sulphur in the nutritional schedule could elevate the yield by 45-49 percent, 68-69 percent and 80-87 percent with the application of sulphur @ 10, 20 and 30 kg/ha, respectively. Higher values were noticed when applied through complex fertilizer (13-33-0-15S) as compared to mixed application through ½AS+½ES. The results of the present finding suggest that the application rate of sulphur could be elevated from 15/ 20 kg S/ha to 30 kg S/ha.

**Uptake of nutrients**

Significant increase in uptake of nutrients viz. nitrogen, phosphorus, potassium, sulphur and zinc was recorded with the application of sulphur as compared to control (Table 3). The higher values for uptake of N (109.7 and 33.9 kg/ha), P (4.06 and 11.10 kg/ha), K (41.0 and 41.5 kg/ha), S (12.40 and 2.67 kg/ha) and Zn (0.28 and 1.29 kg/ha), respectively by grain and straw were observed under $NPKS_{30}$ supplied through complex fertilizer (13-33-0-15S), whereas minimum values were observed under $N_0P_0K_0S_0$. Significant increase in uptake of

**Table 3. Effect of different sources and levels of sulphur on nutrient uptake, protein and oil content of soybean**

| Treatments | Uptake of nutrients (kg/ha) | | | | | Protein | Oil |
|---|---|---|---|---|---|---|---|
| | N | P | K | S | Zn | (%) | (%) |
| | | | | *Grain* | | | |
| $NPKS_0$ | 56.0 | 1.84 | 20.7 | 4.38 | 0.08 | 34.8 | 17.80 |
| $NPKS_{10}$ (½AS+½ES) | 76.2 | 2.62 | 29.0 | 6.47 | 0.13 | 35.1 | 19.61 |
| $NPKS_{20}$ (½AS+½ES) | 92.1 | 3.31 | 34.5 | 7.93 | 0.20 | 36.7 | 19.84 |
| $NPKS_{30}$ (½AS+½ES) | 102.9 | 3.78 | 30.2 | 9.50 | 0.24 | 38.2 | 20.08 |
| $NPKS_{10}$ (13-33-0-15S) | 80.8 | 2.83 | 29.6 | 6.83 | 0.18 | 36.4 | 19.61 |
| $NPKS_{20}$ (13-33-0-15S) | 96.6 | 3.43 | 34.5 | 9.03 | 0.22 | 38.6 | 19.91 |
| $NPKS_{30}$ (13-33-0-15S) | 109.7 | 4.06 | 41.0 | 12.40 | 0.28 | 39.3 | 20.19 |
| $N_0P_0K_0S_0$ | 50.0 | 1.53 | 18.8 | 3.69 | 0.07 | 33.6 | 17.70 |
| SEm ± | 0.26 | 0.019 | 1.57 | 0.07 | 0.001 | 0.06 | 0.012 |
| **CD 5%** | **0.77** | **0.056** | **4.62** | **0.21** | **0.002** | **0.16** | **0.036** |
| | | | | *Straw* | | | |
| $NPKS_0$ | 15.3 | 1.57 | 16.9 | 1.31 | 0.48 | 7.6 | - |
| $NPKS_{10}$ (½AS+½ES) | 22.1 | 2.28 | 24.5 | 1.84 | 0.70 | 8.1 | - |
| $NPKS_{20}$ (½AS+½ES) | 27.7 | 2.86 | 32.4 | 2.22 | 0.91 | 8.7 | - |
| $NPKS_{30}$ (½AS+½ES) | 31.4 | 10.69 | 38.5 | 2.46 | 1.18 | 9.2 | - |
| $NPKS_{10}$ (13-33-0-15S) | 23.5 | 2.36 | 26.3 | 1.93 | 0.80 | 8.6 | - |
| $NPKS_{20}$ (13-33-0-15S) | 28.2 | 2.86 | 33.0 | 2.27 | 1.01 | 9.1 | - |
| $NPKS_{30}$ (13-33-0-15S) | 33.9 | 11.10 | 41.5 | 2.67 | 1.29 | 9.6 | - |
| $N_0P_0K_0S_0$ | 13.0 | 1.38 | 15.3 | 1.14 | 0.42 | 6.9 | - |
| SEm ± | 0.16 | 1.75 | 0.32 | 0.01 | 0.007 | 0.04 | - |
| **CD 5%** | **0.48** | **5.15** | **0.94** | **0.03** | **0.022** | **0.13** | - |

phosphorus and sulphur in black gram consequent to sulphur fertilization has earlier been documented (Singh and Singh, 2004).

**Protein and oil content**

Oil content in soybean seed increased with increasing doses as well as sources of sulphur. The recommended NPK fertilization increased the oil content in seed only by 0.6 percent whereas further inclusion of sulphur resulted in increase to the extent of 11 to 14 percent. The highest oil content (20.19 %) was recorded in case of $NPKS_{30}$ supplied through complex fertilizer (13-33-0-15S). Singh and Sahu (1986) reported an increase in oil content in oilseeds by increasing levels of sulphur application as a result of increase in glycosides, which on hydrolysis increased oil component. Aulakh *et al.* (1990) and Vishwakarma *et al.* (1999) also observed that oil content of soybean seeds increased with varying sources of S application.

Significant increase in protein content was recorded with increasing doses as well as sources of sulphur application in soybean seed and straw both. Maximum protein content in soybean seed and straw accounting to 39.25 and 9.56% respectively was noticed in case of $NPKS_{30}$ applied through complex fertilizer (13-33-0-15S). Mishra and Agrawal (1994) and Vishwakarma *et al.* (1999) noticed that protein content in seeds of soybean was increased with levels as well as sources of sulphur application. Similarly, an increase in protein content of black gram was noticed with addition of sulphur (Singh and Singh 2004).

**Soil properties**

The doses as well as sources of sulphur did not reveal any significant difference in soil pH, EC, organic carbon and available nitrogen after harvest (Table 4). However, significant increase in available $P_2O_5$, $K_2O$, S and DTPA extractable Zn in the soil was recorded with doses and sources of sulphur as compared to control. The maximum values of all the nutrients were noticed in case of complex fertilizer (13-33-0-15S) whereas the lowest values were recorded under control.

**Table 4. Effect of different sources and levels of sulphur on pH, EC and available nutrients status of the soil after harvest of soybean**

| Treatments | pH | EC (dS/m) | O. C. (%) | N | $P_2O_5$ | $K_2O$ | S | Zn (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| | | | | (kg/ha) | | | | |
| $NPKS_0$ | 7.8 | 0.38 | 0.61 | 221.2 | 13.6 | 480 | 7.82 | 1.89 |
| $NPKS_{10}$ (½AS+½ES) | 7.8 | 0.40 | 0.55 | 212.8 | 13.8 | 476 | 7.96 | 2.05 |
| $NPKS_{20}$ (½AS+½ES) | 7.8 | 0.45 | 0.56 | 214.2 | 14.2 | 507 | 8.23 | 2.22 |
| $NPKS_{30}$ (½AS+½ES) | 7.7 | 0.43 | 0.58 | 219.8 | 17.5 | 520 | 8.55 | 2.36 |
| $NPKS_{10}$ (13-33-0-15S) | 7.8 | 0.43 | 0.59 | 220.4 | 15.7 | 491 | 8.10 | 2.12 |
| $NPKS_{20}$ (13-33-0-15S) | 7.8 | 0.44 | 0.53 | 217.0 | 18.0 | 513 | 8.47 | 2.29 |
| $NPKS_{30}$ (13-33-0-15S) | 7.8 | 0.43 | 0.60 | 222.6 | 19.4 | 522 | 8.80 | 2.79 |
| $N_0P_0K_0S_0$ | 7.8 | 0.40 | 0.61 | 208.6 | 12.4 | 466 | 5.57 | 1.65 |
| SEm ± | 0.01 | 0.01 | 0.01 | 1.91 | 0.26 | 2.36 | 0.04 | 0.049 |
| **CD 5%** | NS | NS | NS | NS | 0.76 | 6.95 | 0.11 | 0.145 |

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# Efficacy of Chemical and Biological Seed Dressers and Host Resistance in the Management of Collar Rot of Soybean Caused by *Sclerotium rolfsii* Sacc.

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Soybean (*Glycine max* (L.) Merrill) is affected by many soil borne diseases. Of these, collar rot caused by *Sclerotium rolfsii* Sacc. is gaining a serious status. In Karnataka, it is prevalent in soybean growing areas under rainfed ecosystem. Management of soil borne diseases incited by species of *Sclerotium, Rhizoctonia* and *Fusarium* through one approach is quite difficult (Anahosur, 2001). Hence various seed dressing fungicides and bio-agents were evaluated *in vitro* and further the effective ones *in vivo* singly and in combinations against this disease. Sixty-four soybean genotypes were also evaluated for resistance against *S. rolfsii*.

Seven systemic fungicides (Table 1) were evaluated under *in vitro* condition against *S. rolfsii* following poisoned food technique (Zentmeyer, 1955). Radial growth was measured when the growth in control plates reached 90 mm diameter and percent inhibition of mycelial growth over control was calculated using the formula of Vincent (1927).

Sterilized soil: sand: FYM (1:1:0.5) was uniformly mixed with four percent *S. rolfsii* inoculum multiplied on sand-corn meal medium and filled in 40cm x 30 cm sized pot. Seeds treated with fungicides, bio-agents and their combinations were sown in eight pots @ 10 seeds per pot. Pots were regularly watered and observations were recorded on 7$^{th}$ and 20$^{th}$ day after sowing for pre- and post-emergence seedling mortality and was converted as per cent seedling mortality.

Sixty-four genotypes were screened under glass house in pot culture (Agrawal and Kotasthane, 1971) as also described earlier. Surface sterilized seeds were sown @ 10 seeds per pot separately. The pots devoid of inoculum served as control. Each treatment was replicated twice. Seedling mortality was recorded on 7$^{th}$ and 20$^{th}$ day after sowing. On the basis of percent seedling mortality the genotypes were categorized as Resistant (0-10 %), Moderately Resistant (11-30 %), Moderately Susceptible (31-70 %) and Susceptible (71-100%).

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Five fungal *viz*., *Trichoderma viride* Pers ex. Fr, *Trichoderma harzianum* Rifai, *Gliocladium virens* Millar, *Trichoderma koningii* Oudem and *Trichoderma pseudokoningii* Rifai and four bacterial *viz*., *Pseudomonas fluorescence* Migula, *Pseudomonas striata, Bacillus subtilis* Cohn Emend Pras and *Bradyrhizobium japonicum* antagonists were evaluated against *S. rolfsii* under *in vitro* condition by adopting the procedure given by Huang and Hoes (1976). Twenty milliliter of sterilized and cooled potato dextrose agar was poured into sterilized Petriplates. Pathogens were inoculated at one side and antagonist at exactly opposite side of the same plate leaving 3-4 cm gap. In case of bacterial antagonist, two mycelial discs of pathogen were inoculated and bacterial antagonists was streaked in the centre of the plate. Radial growth of the pathogen was measured when the growth in control plate reached 90 mm diameter. Percent inhibition over control was worked out using the equation given by Vincent (1927).

Fungicides and bio-agents found effective under *in vitro* studies were further evaluated alone and combination as seed dressers using the susceptible variety JS 335 in pot culture under glasshouse condition.

***In vitro* evaluation of fungicides**

All the fungicides significantly inhibited the mycelial growth, which increased with increase in concentrations of fungicides. In systemic fungicides, cent percent inhibition was recorded in carboxin, carbendazim (63 %) + mancozeb (12 %) and propiconazole at all the concentrations. Least percent inhibition was recorded in carbendazim at 0.05 percent followed by thiophanate methyl at 0.05 percent followed by thiophanate methyl at 0.05 and 0.1 percent concentrations. Among the non-systemic fungicides, cent percent inhibition was recorded in thiram at all the concentrations. Chlorothalonil and mancozeb were found next best at 0.2 and 0.3 percent concentrations. Least inhibition was observed in chlorothalonil followed by zineb at 0.1 percent concentration (Table 1). Many workers have reported the effectiveness of carboxin against *S .rolfsii* (Kulkarni *et al*., 1986; El-Wakil and Ghonim, 2000). Johnson and Subramanyam (2000) reported least mycelial inhibition in carbendazim and chlorothalonil.

***In vitro* evaluation of bio-agents**

Among the five fungal and four bacterial antagonists tested, *Trichoderma harzianum* was most effective (79.03 %) followed by *T. viride* (69.90 %) and *G. virens* (65.50 %), all being on par with each other. Similarly, there was no significant difference between *T. koningii* (45.93 %) and *T. pseudokoningii* (43.40%). All the four bacterial antagonists were not much effective. *Pseudomonas fluorescence* exhibited 31.30 percent inhibition and *Bradyrhizobium japonicum* (0.00%) was least effective (Table 2). Pushpavati and Rao (1998) and Iqbal *et al*. (1995) have also recorded maximum inhibition of *S. rolfsii* by *T. harziamum*. These results are also in conformity with Upadhyay and Mukhopadhyay (1983) who reported that *T. harzianum* frequently coiled around the aerial hyphae of *S. rolfsii*. Sometimes it produces haustoria like structures, which enter the mycelium and disorganize the protoplast content, finally causing lysis of fungus. The efficacy of *T. viride* against *S. rolfsii* was reported by Karthikeyan (1996), which may be due to the production of antibiotic (viridin) as reported by Brain (1951).

**Disease management through chemical and biological seed treatment**

Except *Pseudomonas fluorescence* all other seed treatment with fungicides and bio-agents could significantly reduce the pre-emergence mortality due to collar rot (Table 3). Lowest pre-emergence seedling mortality (5.00 %) was recorded in carboxin + *T. harzianum* followed by 11.70 percent in carboxin + *T. viride* and 12.00 percent in thiram + *T. harzianum*, all being at par among themselves. All the treatments reduced the post-emergence seedling mortality significantly and were on par with each other. Uma Singh and Thapliyal (1998) observed maximum reduction in pre-emergence seedling

**Table 1. Percent inhibition of mycelial growth of *S. rolfsii* by different systemic and non-systemic fungicides**

| **Fungicides** | **Growth inhibition (%)** | | | **Mean** |
|---|---|---|---|---|
| | **Concentration** | | | |
| ***Systemic fungicides*** | **0.05 %** | **0.10 %** | **0.20%** | |
| Carboxin | 100.00 | 100.00 | 100.00 | 100.00 |
| | (10.04) | (10.04) | (10.04)* | (10.04) |
| Carbendazim | 0.37 | 24.03 | 94.76 | 39.72 |
| | (1.15) | (4.99) | (9.78) | (5.30) |
| Benomyl | 45.50 | 67.33 | 90.33 | 67.72 |
| | (6.81) | (8.09) | (9.55) | (8.15) |
| Thiophanate methyl | 1.10 | 12.20 | 36.23 | 16.51 |
| | (1.38) | (3.62) | (6.10) | (3.70) |
| Fosetyl aluminium | 10.70 | 46.60 | 89.96 | 49.08 |
| | (3.41) | (6.89) | (9.54) | (6.71) |
| Carbendazim (63%) + | 100.00 | 100.00 | 100.0 | 100.00 |
| Mancozeb (12%) | (10.04) | (10.04) | (10.04) | (10.04) |
| Propiconazole | 100.00 | 100.00 | 100.00 | 100.00 |
| | (10.04) | (10.04) | (10.04) | (10.04) |
| Mean | 51.00 | 64.30 | 87.32 | |
| | (6.12) | (7.67) | (9.27) | |
| | **A (fungicide)** | **B (concentration)** | **A x B** | |
| SEm ± | 0.02 | 0.04 | 0.07 | |
| **CD at 1%** | **0.07** | **0.11** | **0.20** | |
| ***Non systemic fungicides*** | **Concentration** | | | **Mean** |
| | **0.1%** | **0.2%** | **0.3%** | |
| Thiram | 100.00 | 100.00 | 100.00 | 100.00 |
| | (90.00) | (90.00) | (90.00)** | (90.00) |
| Mancozeb | 63.70 | 72.60 | 86.33 | 74.21 |
| | (53.37) | (58.41) | (64.97) | (58.91) |
| Captan | 48.00 | 59.33 | 67.05 | 58.12 |
| | (43.11) | (50.38) | (54.90) | (49.46) |
| Zineb | 38.90 | 43.00 | 47.42 | 43.10 |
| | (38.80) | (42.27) | (43.53) | (41.50) |
| Chlorothalonil | 34.76 | 73.3 | 94.03 | 67.36 |
| | (37.49) | (58.84) | (76.81) | (57.73) |
| Mean | 57.07 | 67.70 | 78.90 | |
| | (52.57) | (59.98) | (69.48) | |
| | **A (fungicide)** | **B (concentration)** | **A x B** | |
| SEm ± | 0.36 | 0.30 | 0.62 | |
| **CD at 1%** | **1.42** | **1.23** | **2.46** | |

** √ x +1 transformed values; ** Arcsine transformed value*

**Table 2. Effect of bio-control agents on mycelial growth of *S. rolfsii***

| Bio-agents | Mycelial growth inhibition (%) |
|---|---|
| *Gliocladium virens* | 65.50 (8.35)* |
| *Trichoderma harzianum* | 79.03 (8.90) |
| *Trichoderma viride* | 69.90 (8.42) |
| *Trichoderma koningii* | 45.93 (6.84) |
| *Trichoderma pseudokoningii* | 43.40 (6.69) |
| *Pseudomonas fluorescens* | 31.3 (5.65) |
| *Bradyrhizobium japonicum* | 0.00 (1.00) |
| *Pseudomonas striata* | 1.48 (1.56) |
| *Bacillus subtilis* | 1.48 (1.56) |
| SEm (±) | 0.28 |
| **CD at 1%** | **1.16** |

* $\sqrt{x+1}$ *transformed values*

**Table 3. Effect of fungicides and bio-agents as seed dressers on seedling mortality of soybean caused by *S. rolfsii***

| Treatments | Per cent seedling mortality | | |
|---|---|---|---|
| | Pre-emergence | Post-emergence | Total |
| $T_1$- Carboxin @ 1 g/kg seed | 19.00 (4.50) | 0.17 (1.04)* | 19.17 |
| $T_2$- Carboxin @ 2 g/kg seed | 14.50 (3.93) | 0.00 (1.00) | 14.50 |
| $T_3$- Thiram @ 2 g/kg seed | 24.00 (5.09) | 0.50 (1.21) | 24.50 |
| $T_4$- Thiram @ 3 g/kg seed | 20.00 (4.60) | 0.30 (1.26) | 20.30 |
| $T_5$- Mancozeb @ 2 g/kg seed | 35.00 (6.00) | 1.00 (1.4) | 36.00 |
| $T_6$- Mancozeb @ 3 g/kg seed | 29.50 (5.60) | 0.50 (1.22) | 30.00 |
| $T_7$- *Trichoderma viride* @ 6 g/kg seed | 30.00 (5.54) | 0.30 (1.26) | 30.30 |
| $T_8$- *T. harzianum* @ 6 g/kg seed | 23.00 (4.90) | 0.30 (1.26) | 23.30 |
| $T_9$- *Pseudomonas fluorescens* @ 10 /kg seed | 68.40 (8.27) | 1.85 (1.69) | 70.25 |
| $T_{10}$- $T_1$ + $T_7$ | 11.70 (3.60) | 0.00 (1.00) | 11.70 |
| $T_{11}$- $T_1$ + $T_8$ | 5.00 (2.44) | 0.00 (1.00) | 5.00 |
| $T_{12}$- $T_1$ + $T_9$ | 30.00 (5.54) | 0.17 (1.04) | 30.17 |
| $T_{13}$- $T_3$ + $T_7$ | 25.00 (5.00) | 0.00 (1.00) | 25.00 |
| $T_{14}$- $T_3$ + $T_8$ | 12.00 (3.62) | 0.00 (1.00) | 12.00 |
| $T_{15}$- $T_3$ + $T_9$ | 28.00 (5.27) | 0.33 (1.15) | 28.33 |
| $T_{16}$- $T_5$ + $T_7$ | 29.75 (5.66) | 0.00 (1.00) | 29.75 |
| $T_{17}$- $T_5$ + $T_8$ | 22.00 (4.80) | 0.00 (1.00) | 22.00 |
| $T_{18}$- $T_5$ + $T_9$ | 37 (6.40) | 0.35 (1.14) | 37.50 |
| $T_{19}$- Control | 73.00 (8.62) | 13.00 (3.70) | 86.00 |
| SEm ± | 0.32 | 0.06 | |
| **CD at 1 %** | 1.30 | 0.28 | |

* $\sqrt{x+1}$ *transformed values*

mortality with seed treatment of vitavax 200 WP + *T. harzianum* and vitavax 200 WP + *G. virens* followed by vitavax 200 WP, thiram, thiram + *T. harzianum* and *G. virens*. Combination of carboxin with *T. harzianum* was found best in integrated management of *Corticium rolfsii*. This might be due to the tolerance of *T. harzianum* to carboxin (Montealegue and Henrique, 1990).

**Identification of resistant sources**

None of the 64 tested genotypes exhibited resistant or moderately resistant reaction. However, only fifteen genotypes *viz*., VLS 57, NRC 5, DSb 5, VLS 2, SL 659, PKS 9, AMS 2001-1, DSb 7, DSb 3, 95-14, JS (SH) 96-31, PK 416, SL518, JS 335 and Alankar showed moderately susceptible reaction. Remaining forty-nine genotypes showed susceptible reaction. Earlier workers also could not find any soybean genotype resistant to *S. rolfsii* (Agrawal and Kotasthane, 1971 and Uma Singh and Thapliyal, 1999).

The results suggest that among fungicides carboxin, carbendazim (63%) + mancozeb (12%), propiconazole and thiram and among the bio-agents, *Trichoderma harzianum* and *T. viride* were effective in inhibiting the growth of *S. rolfsii*. Seed treatment with carboxin (1g/kg seed) + *T. harzianum* (6 g/kg seed) showed least pre- and post- emergence seedling mortality.

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# Two Genotypes of Soybean as Promising Source of Resistance to Rust Caused by *Phakopsora pachyrhizi* Syd.

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Soybean rust incited by *Phakopsora pachyrhizi* Syd. is one of the major diseases of soybean and is reported to cause an yield loss from 20 to 80 percent (Bromfield 1976). During *kharif* 1994, the rust appeared in epiphytotic form in different soybean growing areas and caused substantial losses. Then onwards it is appearing every year in epiphytotic form in northern parts of Karnataka, Maharashtra and Madhya Pradesh (Patil *et al*. 1997). Though the effective fungicides have been identified for its control, their continuous use may pose the problem of development of resistance in the rust pathogen to these fungicides (Patil and Anahosur 1988). Development of resistant genotypes is of prime importance in the management of soybean rust disease. Most of the soybean genotypes identified or developed by the earlier workers found moderately resistant to rust (Singh and Thapliyal 1977, Yang, 1978, Bromfield *et al*., 1980 and Bharati, 1989). Chan (1976) opined that solely breeding cannot solve soybean rust problem until a highly resistant or immune cultivar is available. The attempt made since *kharif* 1995 to 2001 to screen the soybean varieties/germplasm lines has yielded only few genotypes with moderate amount of resistance (Patil and Basavaraja, 1997). Hence, an attempt was made to screen 982 soybean genotypes supplied by the National Research Centre for Soybean, Indore during *kharif* 2002 and 2003 at Research and Development Unit, Ugar Khurd, Belgaum district, Karnataka, a hot spot for soybean rust disease. The genotypes were sown in single rows of five-metre length and scoring was done at 75, 85 and 95 days after sowing depending on maturity duration. For scoring, the standard IWGSR (International Working Group on Soybean Rust) three-digit scientific notation was adopted (Shanmugasundaram 1977). Scoring was also done under 1-9 grade scale given by Mayee and Datar (1986)

The results indicated that, only two genotypes *viz*., EC 241778 and EC 241780 have showed resistant reaction (3 grade) as most of top leaves were found free from the rust pustules (311) with medium to non sporulating light pustule density on the middle (221 and 222) and

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lower (121 and 122) leaves. Six genotypes *viz*., EC 325115, EC 251378, EC 389149, EC 432536, EC 241760 and EC 333917 have shown moderately resistant reaction (5 grade) as their bottom third leaves produced medium to heavy medium sporulating pustules (132 and 142). Sixty-eight genotypes indicated susceptible reaction (7 grade) by producing light to medium density highly sporulating pustules on middle (223 and 233) and bottom leaves (123 and 133). All the remaining 906 genotypes have showed highly susceptible (9 grade) reaction as their bottom, middle and upper leave were covered heavily with highly sporulating pustules (143, 243 and 343). The resistant genotypes are characterized by production of rectangular reddish brown non-sporulating pustules where as moderately resistant genotypes produced rectangular reddish brown medium sporulating pustules. The susceptible genotypes produced light to medium density grey coloured sporulating pustules where as highly susceptible genotypes produced high-density grey coloured highly sporulating pustules on all the leaves and premature defoliation was much common in all the highly susceptible genotypes. The two soybean genotypes, EC 241778 and EC 241780, which have shown resistant reaction, can be used in breeding programme for rust resistance.

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# Screening of Elite Soybean Lines for Resistance against Stem-fly (*Melanagromyza sojae* Zehntner)

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**Key words:** Soybean, stem fly, host plant resistance

Stem fly (*Melanagromyza sojae* Zehnt.) is a serious insect-pest of soybean in India. In the early growth stage of the crop, infestation of stem fly results in death of seedlings where as in grown up plants reduced vigor is observed. The infestation reduces grain yield by 25-30 percent (Kundu and Srivastava 1991). Soybean varieties show varying degrees of stem fly infestation owing to host plant resistance. Present studies include the results of three year's field experiments aimed at screening of elite soybean lines for their resistance against major insect-pests.

A total of 107 elite soybean lines were planted in randomized block design with three replications along with national check - Bragg, susceptible check - PK 1029 and high yielding check - MACS 124 (1998) and MACS 450 (2000 and 2002). Each plot consisted of three rows of 3-meter length with 45 cm and 5 cm distance between and within rows, respectively. Data were recorded on total plant height and length of stem tunneled by stem fly. Stem tunneling was expressed in percentage and angular transformed values of percentages were used for ANOVA. Soybean lines were categorized as highly resistant (HR), Resistant (R), Moderately Resistant (MR), Low Resistant (LR), Susceptible (S) and Highly Susceptible (HS) for resistance as per AICRPS method (2001) based on Least Square Difference (LSD) values at 5 percent and 1 percent probability levels and economic threshold level for stem tunneling i.e. 26 percent. Seed yield was recorded in g on net plot basis (2.5 m x 1.35 m) and converted into kg/ha by multiplication factor 2.936.

ANOVA for stem tunneling (arcsine values) and grain yield indicated significant differences among the elite lines tested. Susceptible check var. PK 1029- recorded 34.75 percent, 30.66 percent and 38.52 percent stem tunneling in the years 1998, 2000 and 2002, respectively. These values were above the economic threshold level value (i. e. 26%) determined by Venkatesan and Kundu (1994). Thus, the data recorded on stem tunneling can reliably be categorized for resistance.

Out of 107 elite lines tested, 18 lines were highly resistant (HR) to stem fly

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**Table 1. Categorization of elite soybean lines for resistance against stem fly and yield performance in the year 1998**

| Name of the line | Stem tunneling (%)* | Category | Yield (kg/ha) |
|---|---|---|---|
| DS 9801 | 22.65 (28.36) | LR | 2626** |
| DS 9802 | 15.69 (23.33) | MR | 2412 |
| Himso 1577 | 33.87 (35.45) | HS | 2031 |
| Himso 1578 | 06.36 (14.53) | HR | 2764 |
| Himso 1579 | 26.97 (31.14) | S | 2652 |
| JS 92-12 | 04.80 (12.60) | HR | 2903 |
| JS 92-14 | 24.63 (29.69) | S | 1882 |
| JS 92-22 | 10.64 (20.62) | MR | 2822 |
| JS(SH) 93-01 | 08.93 (15.40) | HR | 3463** |
| JS(SH) 93-37 | 07.81 (16.19) | HR | 3169** |
| JS(SH) 93-44 | 14.72 (22.52) | S | 3017** |
| JS(SH) 93-48 | 07.97 (16.39) | HR | 2881 |
| LSb 3 | 15.27 (22.93) | MR | 3028** |
| MACS 493 | 17.23 (24.41) | MR | 2203 |
| MACS 565 | 28.05 (31.91) | HS | 2551 |
| MACS 569 | 10.12 (18.39) | R | 2907 |
| MACS 629 | 15.83 (23.41) | MR | 2646 |
| MAUS 61 | 12.02 (20.17) | MR | 3380** |
| MAUS 62 | 14.75 (22.56) | MR | 3454** |
| MAUS 63 | 11.11 (19.14) | MR | 3431** |
| MAUS 68 | 12.79 (20.92) | MR | 3614** |
| NRC 40 | 46.51 (42.97) | HS | 1836 |
| NRC 41 | 12.99 (21.13) | MR | 3203** |
| NRC 42 | 16.34 (23.69) | MR | 2654 |
| NRC 43 | 39.37 (38.79) | HS | 1542 |
| NRC 44 | 22.46 (28.20) | LR | 1673 |
| PK 1223 | 13.64 (21.68) | MR | 1756 |
| PK 1225 | 21.77 (27.75) | LR | 2780 |
| PK 1228 | 27.25 (31.42) | S | 1812 |
| PK 1229 | 20.12 (26.59) | LR | 1492 |
| PK 1243 | 31.80 (34.25) | HS | 2359 |
| RAUS 3 | 14.82 (22.51) | MR | 3278** |
| RAUS 4 | 16.40 (23.90) | MR | 2983** |
| SL 295 | 14.43 (22.23) | MR | 2204 |
| SL 432 | 21.21 (27.40) | LR | 3447** |
| SL 459 | 12.15 (20.32) | MR | 2178 |

| | | | |
|---|---|---|---|
| | 22.77 (28.45) | LR | 2652 |
| TS 98-1 | | | |
| TS 98-21 | 2.20 ( 8.50) | HR | 3255** |
| TS 98-91 | 7.05 (15.30) | HR | 3572** |
| UGM 47 | 3.57 (10.56) | HR | 2447 |
| VLS 32 | 41.23 (39.88) | HS | 2396 |
| VLS 53 | 34.03 (35.37) | HS | 1664 |
| Bragg (National check) | 34.44 (35.89) | HS | 2412 |
| MACS 124 (High yielding check) | 8.36 (16.79) | HR | 3352 |
| PK 1029 (Susceptible check) | 34.75 (36.12) | HS | 2947 |
| Mean | 18.20 (24.60) | | 2660 |
| **LSD (P=0.05)** | **(5.34)** | | |
| **LSD (P=0.01)** | **(7.08)** | | **423** |
| CV (%) | 13.36 | | 9.81 |

**Arcsine values are given in parentheses; **At par with the high yielding check variety of the respective year.*

**Table 2. Categorization of elite soybean lines for resistance against stem fly and yield performance in the year 2000**

| Name of the line | Stem tunneling (%)* | Category | Yield (kg/ha) |
|---|---|---|---|
| AMS 97-1 | 14.40 (22.27) | HR | 2256 |
| AMS 97-2 | 23.29 (28.84) | MR | 2225 |
| DS 97-11 | 26.13 (30.72) | LR | 2198 |
| DS 97-12 | 24.44 (29.62) | MR | 2226 |
| DSb 5 | 10.51 (18.90) | HR | 2717** |
| Himso 1587 | 29.03 (32.59) | LR | 1982 |
| Himso 1588 | 46.83 (43.18) | HS | 2029 |
| HIS 01 | 30.73 (33.55) | LR | 2510 |
| JS 94-65 | 16.47 (23.90) | HR | 1850 |
| JS 94-66 | 25.50 (30.31) | MR | 2726** |
| JS 94-67 | 17.71 (24.89) | R | 2440 |
| JS(SH) 94-167 | 21.74 (27.78) | MR | 2446 |
| JS(SH) 95-26 | 29.05 (32.54) | LR | 1972 |
| KB 221 | 15.76 (23.39) | HR | 2646** |
| KB 222 | 18.15 (25.22) | R | 2138 |

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|---|---|---|---|
| MACS 666 | 19.35 (26.08) | MR | 2392 |
| MACS 730 | 27.80 (31.80) | LR | 2773** |
| MACS 740 | 25.67 (30.40) | MR | 2598** |
| MAUS 62-2 | 26.01 (30.62) | LR | 2814** |
| MAUS 64-1 | 13.98 (21.97) | HR | 2522 |
| MAUS 81 | 27.92 (31.83) | LR | 2492 |
| NRC 51 | 19.98 (26.52) | MR | 2482 |
| NRC 52 | 16.30 (23.75) | HR | 2248 |
| NRC 53 | 21.40 (27.50) | MR | 2278 |
| PK 1274 | 26.00 (30.64) | LR | 1648 |
| PK 1283 | 37.78 (37.88) | HS | 1509 |
| PK 1284 | 45.86 (42.61) | HS | 1719 |
| SL 328 | 44.28 (41.67) | HS | 1667 |
| SL 428 | 30.72 (33.63) | LR | 979 |
| SL 603 | 45.70 (42.49) | HS | 684 |
| TS 2000-129 | 31.84 (34.37) | LR | 2221 |
| TS 2000-20 | 26. 26 (30.80) | LR | 2491 |
| VLS 54 | 29.87 (33.04) | LR | 1658 |
| Bragg (National check) | 31.89 (34.34) | LR | 1575 |
| MACS 450 (High yielding check) | 16.25 (23.78) | HR | 2932 |
| PK 1029 (Susceptible check) | 30.66 (33.56) | LR | 2241 |
| Mean | 26.20 (30.43) | | 2194 |
| **LSD (P=0.05)** | **(4.57)** | | |
| **LSD (P=0.01)** | **(6.07)** | | **338** |
| CV (%) | 9.21 | | 9.46 |

**Arcsine values are given in parentheses; **At par with the high yielding check variety of the respective year.*

**Table 3. Categorization of elite soybean lines for resistance against stem fly and yield performance in the year 2002**

| Name of the line | Stem tunneling (%)* | Category | Yield (kg/ha) |
|---|---|---|---|
| AMS 2001-1 | 14.24(22.12) | HR | 0809 |
| DS 9909 | 34.08 (35.73) | LR | 1258 |
| 3 DSb 6 | 29.26 (32.64) | LR | 1548 |
| Himso 1598 | 25.41 (30.26) | MR | 1496 |
| Himso 1599 | 19.27 (25.94) | MR | 0877 |

| | | | |
|---|---|---|---|
| JS 95-60 | 35.55 (36.55) | LR | 0729 |
| JS 96-31 | 24.43 (29.54) | MR | 1357 |
| JS(SH) 96-16 | 23.96 (29.30) | MR | 1618 |
| MACS 757 | 27.28 (31.46) | MR | 1843** |
| MACS 869 | 24.72 (29.65) | MR | 1357 |
| MACS 871 | 10.20 (18.52) | HR | 1490 |
| MAUS 2 | 26.44 (30.85) | MR | 0920 |
| MAUS 30 | 29.06 (32.58) | LR | 1207 |
| MAUS 162 | 14.40 (22.15) | HR | 1744 |
| MAUS 164 | 18.84 (25.72) | MR | 1766 |
| MRSB 342 | 25.80 (30.47) | MR | 1505 |
| NRC 59 | 30.90 (33.70) | LR | 1436 |
| NRC 61 | 28.97 (32.56) | LR | 1145 |
| PK 1337 | 44.06 (41.57) | HS | 0279 |
| PK 1343 | 53.30 (46.91) | S | 0188 |
| PK 1347 | 51.27 (45.73) | HS | 0114 |
| RKS 9 | 42.14 (40.49) | S | 1997** |
| RKS 12 | 32.19 (34.50) | LR | 0446 |
| SL 633 | 29.00 (32.58) | LR | 1341 |
| SL 637 | 40.31 (39.41) | S | 1529 |
| SL 659 | 26.24 (30.80) | MR | 1354 |
| TS 3 | 20.13 (26.65) | MR | 1255 |
| TS 148-22 | 43.87 (41.47) | S | 1178 |
| UGM 20075 | 9.58 (18.04) | HR | 1187 |
| VLS 57 | 34.20 (35.76) | LR | 1074 |
| VLS 58 | 33.37 (35.26) | LR | 0951 |
| Bragg (National check) | 30.51 (33.48) | LR | 1099 |
| MACS 450 (High yielding check) | 20.94 (27.24) | MR | 1868 |
| PK 1029 (Susceptible check) | 38.52 (38.31) | LR | 1247 |
| Mean | 29.35 (32.40) | | 1210 |
| **LSD (P=0.05)** | **(4.57)** | | |
| **LSD (P=0.01)** | **(6.92)** | | **0231** |
| CV (%) | 11.50 | | 9.38 |

**Arcsine values are given in parentheses; ** At par with the high yielding check variety of the respective year.*

damage. Three lines viz. KB 222, JS 94-67 and MACS 569 were found to be resistant (R). On the basis of categorization, 35, 29, 8 and 14 lines were moderately resistant (MR), low resistant (LR), susceptible (S) and highly susceptible (HS), respectively.

Twenty-five lines recorded higher seed yield than the high yielding check variety of respective year. However, only 7 lines *viz*. DSb 5, JS (SH) 93-01, JS(SH) 93-37, KB 22, MAUS 162, TS 98-21

and TS 98-91 showed higher degree of resistance to stem fly combined with higher seed yield than other lines. These lines have a great promise in hybridization programme. Six lines viz. DS 9801, JS(SH) 93-44, MACS 730, MAUS 62-2, RKS 9 and SL 432 though showed low resistance or susceptibility to stem fly, out yielded the high yielding check variety. These lines possess tolerance against stem fly infestation.

Kundu and Srivastava (1991), Sharma and Shukla (1993), Sharma *et al.* (1994), Kundu *et al*. (1995) and Taware *et al.* (2001) have screened several soybean germplasm lines and varieties for resistance against stem fly and have reported some of them to be promising.

Since stem fly is a major insect-pest of soybean in India, highly resistant and high yielding elite lines reported in the present studies will be useful in breeding for stem fly resistant soybean varieties.

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# Effect of Bio-rational and Chemical Insecticides on Stem Borers and Yield of Soybean [(*Glycine Max* (L.) Merrill]

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**Key words**: Soybean, *Melanagromyza sojae, Obereopsis brevis,* bio-rational, yield

Soybean, *Glycine max* (L.) Merrill, is attacked by 273 insect pests. Among the various insect pests ravaging soybean crop in Northern India, the stem borers, namely stem fly, *Melanagromyza sojae* (Zehntner) and girdle beetle, *Obereopsis brevis* (Swed.) are the real menace. The stem fly infests over 90 percent while girdle beetle may infest up to 80 percent soybean plants in various parts of India. Girdle beetle is reported to reduce the grain yield up to 30.2 percent (Singh and Singh, 1989). A reduction of 78.26 percent in pod number, 85.08 percent in pod weight, 84.30 percent in grain number and 85.35 percent in grain weight has been reported due to stem tunneling at the early stage of the crop growth (Gangrade 1974). So far, only chemical control measures are in vogue to manage the stem borers. In order to find the alternative control measures of stem borers in the *Tarai* region of Uttaranchal, bio-rational insecticides and chemical insecticide were evaluated in the present study.

Field experiment was carried out during *kharif*-2000 at Crop Research Centre, Pantnagar. The experiment was laid out in factorial randomized block design having nine treatments (Table 1) and three replications. Plot size was 5.0 m × 4.2 m to accommodate seven rows. Distance between row-to-row and plant-to-plant was 60 cm and 5 cm, respectively. Soybean varieties, PK 1029 and PK 416, were grown for present study. Treatments were applied at 35 and 64 days after sowing (DAS). The total spray volume used was 600 l/ha. Stem tunneling caused by stem fly, *M. sojae* were recorded on 90 DAS by splitting five plants/replication. Percent tunneling was then calculated on the basis of tunnel length and plant height. Girdle beetle infestation was recorded two middle rows at 1 day before and 1, 3 and 7 days after each spray. At the time of harvest, the yield was recorded from five inner rows by discarding one row on both side and 0.5 m at both the ends of rows. The data was transformed in angular and square root (Snedecor and Cochran 1959) and statistically analyzed. The cost-benefit ratio was computed by using the mean grain yield per hectare.

Considering the mean per cent infested plants, none of the treatments was effective,

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however on PK 1029, Neem seed cake extract (NSKE) 4 percent, cow urine 10 percent and cow dung ash and on PK 416, diflubenzuron 25 WP and NSKE 6 percent showed considerable reduction in stem fly infestation (Table 1). A significant reduction in tunneling percentage in PK 416 was recorded due to two spray of NSKE 6 percent followed by cow urine 20 percent, diflubenzuron 25 WP, NSKE 4 percent and cow urine 10 percent but synthetic chemical insecticide triazophos 40EC was not effective. Shri Ram (1991) and Singh (1994) have also reported the effectiveness of *Neem* seed water extract 4 percent to reduce the stem fly infestation. Chaudhary *et al.* (1981) and Singh and Singh (1989) used granular systemic insecticides against *M. sojae* and found good yield and less tunneling. Venkatesan and Kundu (1994) found that the chemical insecticides were effective for controlling the stem fly.

The percent infestation of girdle beetle, *O. brevis* was observed from 34 to 72 DAS, but it was very low up to 38 DAS (Table 2). After 42 DAS (7 days after spraying), girdle beetle infestation appeared in all the treatments except diflubenzuron 25 WP and triazophos 40 EC and varied significantly from 0.00 to 1.99. Maximum percent infestation was recorded in untreated plot that was 1.93 and 1.99 in PK 1029 and PK 416, respectively. Based on the observation taken after 3 days of second spraying, NSKE 4 percent and cow urine 10 percent on PK 1029 and *B. bassiana (*Dispel) with PK 416 could be considered effective to manage the girdle beetle infestation. At 7$^{th}$ day of second spraying (72 DAS) the minimum infestation was recorded in diflubenzuron 25 WP, NSKE 4 percent, cow urine 20 percent and triazophos 40 EC treated plot in PK 1029, while in PK 416, it was 0.18 in *B. bassiana* treated plots. Bhattacharya *et al.* (1998) observed that triazophos performed better in checking the damage by girdle beetle followed by Neem seed water extract.

Significant differences were observed among the treatments with respect to grain yield. Maximum grain yield 28.95 and 25.06 q/ha was obtained from the plot treated with triazophos 40 EC and minimum yield was in untreated plot that was 18.56 and 14.24 q/ha in PK 1029 and PK 416, respectively (Table 3). Next to triazophos the maximum grain yield was obtained in diflubenzuron WP and *Beauveria bassiana* treated plots. The results were similar to the findings of Bhattacharya *et al.* (1998), who reported that *Bacillus thuringiensis* (Biobit) and triazophos have the similar efficacy. Purwar and Yadav (2003) also observed that triazophos was most effective against *Spodoptera litura* followed by diflubenzuron and *B. bassiana*. Dubey *et al.* (1998) proved that use of triazophos provided maximum profit but the use of microbial agents although reduced the larval population and stem tunneling by *Melanagromyza sojae*, was not profitable due to high cost.

The *B. bassiana*, diflubenzuron, NSKE 4 percent, NSKE 6 percent, cow urine 20 percent, cow urine 10 percent, cow dung ash and triazophos treated plots exhibited 5.34, 8.18, 9.09, 6.59, 7.91, 10.06, 9.09, 10.82 q/ha additional yield over control, respectively in PK 416 cultivar of soybean. Whereas in case of PK 1029 additional grain yield over control was 7.27, 7.97, 5.04, 5.33, 3.93, 5.81, 4.27, 10.39 q/ha in different treatments (Table 3). These results revealed that triazophos had highest grain yield over control in both the cultivars followed by cow urine 10 percent and diflubenzuron 25 WP in PK 416 and PK 1029, respectively.

Highest cost-benefit ratio of 1:17.74 was recorded in case of two sprays of cow urine 10 percent in PK 1029 and 1:31.45 in PK 416. Diflubenzuron 25 WP had the minimum cost-benefit ratio of 1:1.77 in PK 1029 and 1:1.67 in PK 416 with *B. bassiana* due to their high cost. Other treatments that have higher cost-benefit ratio were cow dung ash where it was 1:12.77

**Table 1. Effect of different treatments on stem fly infestation on two cultivars of soybean**

| Treatments (B) | Per cent Infestation after 90 DAS | | | Per cent tunneling after 90 DAS | | |
|---|---|---|---|---|---|---|
| | Varieties (A) | | | Varieties (A) | | |
| | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean |
| Control | 100.00 | 100.00 | 100.00 | 29.80 | 25.55 | 27.68 |
| | (90.00) | (90.00)* | (90.00) | (33.00) | (30.36) | (31.68) |
| *B. bassiana* | 100.00 | 93.33 | 96.67 | 28.18 | 25.37 | 26.78 |
| | (90.00) | (81.15) | (85.57) | (32.01) | (30.04) | (31.03) |
| Diflubenzuron | 100.00 | 80.00 | 90.00 | 21.98 | 12.24 | 17.11 |
| | (90.00) | (68.07) | (79.03) | (28.04) | (20.09) | (24.06) |
| NSKE 4% | 93.33 | 100.00 | 96.67 | 22.38 | 14.57 | 18.48 |
| | (81.15) | (90.00) | (85.57) | (28.03) | (22.44) | (25.23) |
| NSKE 6% | 100.00 | 66.67 | 83.33 | 29.57 | 8.23 | 18.90 |
| | (90.00) | (60.00) | (75.00) | (32.93) | (14.28) | (23.60) |
| Cow Urine 20% | 100.00 | 93.34 | 96.67 | 27.76 | 9.47 | 18.62 |
| | (90.00) | (81.15) | (85.57) | (31.76) | (17.75) | (24.76) |
| Cow Urine 10% | 93.33 | 100.00 | 96.67 | 23.31 | 16.77 | 20.04 |
| | (81.15) | (90.00) | (85.57) | (28.77) | (24.17) | (26.47) |
| Cow dung ash | 93.33 | 86.67 | 90.00 | 30.77 | 19.34 | 25.06 |
| | (81.15) | (72.29) | (76.72) | (33.13) | (25.98) | (29.56) |
| Triazophos 40EC | 100.00 | 86.67 | 93.33 | 22.50 | 20.06 | 21.28 |
| | (90.00) | (72.29) | (81.15) | (28.26) | (25.29) | (26.77) |
| Mean | 98.52 | 89.63 | 94.07 | 26.27 | 16.86 | 21.56 |
| | (88.04) | (78.32) | (83.18) | (30.65) | (23.38) | (27.11) |
| | SEm ± | **CD (P=0.05)** | | SEm ± | **CD (P=0.05)** | |
| A | 2.38 (2.30) | **6.85(6.61)** | | 1.50 | **4.32(3.19)** | |
| B | 5.05(4.87) | **14.55(14.02)** | | 3.19 | **9.18(6.67)** | |
| A*B | 7.16(6.90) | **20.57(9.83)** | | 4.51 | **12.11(9.57)** | |

** Data in parentheses are angular transformed values*

**Table 2. Effect of different treatments on plant infestation (%) by Girdle beetle on two cultivars of soybean**

| Treatments (B) | Before 1 day of first spray (34 DAS) Varieties (A) | | | After 1 day of first spray (36 DAS) Varieties (A) | | | After 3 days of first spray (38 DAS) Varieties (A) | | | After 7 days of first spray (42 DAS) Varieties (A) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean | PK-1029 | PK-416 | Mean |
| Control | 1.15 | 1.70 | 1.43 | 0.93 | 0.40 | 0.67 | 0.46 | 1.26 | 0.86 | 1.93 | 0.40 | 1.17 |
| | (6.16) | (7.43) | (6.80) | (5.47) | (2.97) | (4.22) | (3.18) | (6.29) | (4.74) | (7.92) | (2.11) | (5.01) |
| *B. bassiana* | 0.47 | 0.19 | 0.33 | 0.22 | 0.20 | 0.21 | 0.00 | 0.57 | 0.28 | 0.00 | 1.99 | 0.99 |
| | (3.21) | (1.46) | (2.33) | (1.57) | (1.48) | (1.52) | (0.00) | (3.49) | (1.74) | (0.00) | (8.08) | (4.04) |
| Diflubenzuron | 0.37 | 0.00 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.19 | 0.10 | 0.00 | 0.00 | 0.00 |
| | (2.09) | (0.00) | (1.04) | (0.00) | (0.00) | (0.00) | (0.00) | (1.46) | (0.73) | (0.00) | (0.00) | (0.00) |
| NSKE 4% | 0.30 | 0.20 | 0.25 | 0.30 | 0.45 | 0.37 | 0.51 | 19.33 | 9.92 | 0.30 | 0.26 | 0.28 |
| | (1.80) | (1.49) | (1.65) | (1.80) | (3.13) | (2.47) | (3.35) | (16.53) | (9.94) | (1.80) | (1.69) | (1.75) |
| NSKE 6% | 0.45 | 0.66 | 0.56 | 0.93 | 0.39 | 0.66 | 0.93 | 0.40 | 0.67 | 0.00 | .19 | 0.09 |
| | (2.22) | (3.75) | (2.99) | (5.47) | (2.92) | (4.20) | (5.47) | (2.97) | (4.22) | (0.00) | (1.43) | (0.72) |
| Cow Urine | 0.00 | 0.42 | 0.21 | 0.00 | 0.19 | 0.09 | 0.00 | 0.19 | 0.09 | 0.22 | 0.64 | 0.43 |
| 20% | (0.00) | (2.16) | (1.08) | (0.00) | (1.43) | (0.72) | (0.00) | (1.43) | (0.72) | (1.55) | (3.65) | (2.60) |
| Cow Urine | 0.90 | 0.00 | 0.45 | 0.00 | 0.21 | 0.11 | 1.06 | 0.00 | 0.53 | 0.43 | 0.21 | 0.32 |
| 10% | (4.46) | (0.00) | (2.23) | (0.00) | (1.52) | (0.76) | (5.90) | (0.00) | (2.95) | (3.08) | (1.51) | (2.29) |
| Cow dung ash | 0.00 | 1.28 | 0.64 | 0.61 | 0.21 | 0.41 | 0.95 | 0.41 | 0.68 | 0.23 | 0.22 | 0.22 |
| | (0.00) | (6.49) | (3.24) | (4.48) | (1.51) | (2.99) | (5.56) | (2.13) | (3.84) | (1.58) | (1.54) | (1.56) |
| (Triazophos | 0.60 | 1.35 | 0.97 | 0.00 | 0.40 | 0.20 | 0.25 | 0.40 | 0.33 | 0.00 | 0.00 | 0.00 |
| 40EC | (3.58) | (6.65) | (5.12) | (0.00) | (2.97) | (1.49) | (1.67) | (2.97) | (2.32) | (0.00) | (0.00) | (0.00) |
| Mean | 0.47 | 0.65 | 0.56 | 0.33 | 0.27 | 0.30 | 0.46 | 2.53 | 1.50 | 0.35 | 0.43 | 0.39 |
| | (2.61) | (3.27) | (2.94) | (2.09) | (1.99) | (2.04) | (2.79) | (4.14) | (3.47) | (1.77) | (2.22) | (2.00) |
| | SEm ± | **CD (P=0.05)** | | SEm ± | **CD (P=0.05)** | | SEm ± | **CD (P=0.05)** | | SEm ± | **CD (P=0.05)** | |
| A | 0.09(0.49) | **NS** | | 0.06(0.40) | **NS** | | 1.52(1.36) | **NS** | | 0.07(0.42) | **NS** | |
| B | 0.20(1.03) | **0.57(2.96)** | | 0.13(0.84) | **0.36(0.26)** | | 3.23(2.89) | **NS** | | 0.16(0.90) | **0.45(2.57)** | |
| A*B | 0.28(1.46) | **0.80(4.19)** | | 0.18(1.18) | **NS** | | 0.07(0.42) | **NS** | | 0.22(1.27) | **0.64(3.64)** | |

**Table: Contd…**

| Treatments (B) | Before 1 day of second spray (64 DAS) | | | After 1 day of second spray (66 DAS) | | | After 3 days of second spray (68 DAS) | | | After 7 days of second spray (72 DAS) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Varieties(A) | | | Varieties(A) | | | Varieties(A) | | | Varieties(A) | | |
| | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean | PK 1029 | PK 416 | Mean |
| Control | 0.99 | 1.01 | 1.00 | 1.88 | 1.45 | 1.67 | 1.97 | 1.20 | 1.59 | 1.45 | 3.99 | 2.72 |
| | (5.68)* | (5.71) | (5.69) | (7.78) | (6.22) | (7.00) | (7.98) | (1.49) | (4.74) | (6.74) | (11.46) | (9.10) |
| *B. bassiana* | 7.26 | 0.94 | 4.10 | 0.00 | 0.12 | 0.06 | 0.47 | 0.00 | 0.24 | 0.23 | 0.18 | 0.21 |
| | (10.68) | (4.52) | (7.60) | (0.00) | (0.78) | (0.39) | (2.27) | (0.00) | (1.14) | (1.60) | (1.42) | (1.51) |
| Diflubenzuron | 1.06 | 1.82 | 1.44 | 0.00 | 0.41 | 0.21 | 0.81 | 0.39 | 0.60 | 0.00 | 0.19 | 0.10 |
| | (4.68) | (6.21) | (5.45) | (0.00) | (1.50) | (0.75) | (3.00) | (2.07) | (2.53) | (0.00) | (1.46) | (0.73) |
| NSKE 4% | 0.00 | 2.65 | 1.32 | 1.57 | 0.79 | 1.18 | 0.00 | 1.30 | 0.65 | 0.00 | 0.88 | 0.44 |
| | (0.00) | (7.65) | (3.82) | (5.85) | (2.93) | (4.39) | (0.00) | (5.23) | (2.62) | (0.00) | (4.35) | (2.18) |
| NSKE 6% | 0.25 | 1.00 | 0.62 | 2.01 | 1.35 | 1.68 | 0.25 | 0.20 | 0.22 | 0.45 | 0.20 | 0.33 |
| | (1.64) | (5.59) | (3.62) | (4.73) | (4.30) | (4.51) | (1.64) | (1.48) | (1.56) | (2.22) | (1.48) | (1.85) |
| Cow Urine 20% | 0.88 | 0.82 | 0.85 | 1.27 | 0.64 | 0.96 | 1.52 | 0.82 | 1.17 | 0.00 | 1.05 | 0.52 |
| | (4.30) | (4.24) | (4.27) | (5.17) | (2.58) | (.88) | (7.02) | (4.24) | (5.63) | (0.00) | (4.75) | (2.37) |
| Cow Urine 10% | 0.22 | 1.88 | 1.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.41 | 0.21 | 0.22 | 0.21 | 0.22 |
| | (1.54) | (6.35) | (3.95) | (0.00) | (0.00) | (0.00) | (0.00) | (2.13) | (1.06) | (1.52) | (1.51) | (1.52) |
| Cow dung ash | 0.98 | 1.06 | 1.02 | 0.44 | 0.33 | 0.39 | 0.23 | 0.22 | 0.22 | 0.23 | 0.21 | 0.22 |
| | (4.45) | (4.80) | (4.63) | (2.19) | (1.89) | (2.04) | (1.59) | (1.54) | (1.56) | (1.59) | (1.51) | (1.55) |
| Triazophos 40EC | 0.00 | 0.35 | 0.17 | 0.41 | 0.21 | 0.31 | 0.00 | 0.23 | 0.11 | 0.00 | 0.23 | 0.11 |
| | (0.00) | (3.26) | (1.63) | (3.00) | (1.50) | (2.25) | (0.00) | (1.58) | (0.79) | (0.00) | (1.58) | (0.79) |
| Mean | 1.29 | 1.28 | 1.29 | 0.84 | 0.59 | 0.71 | 0.58 | 0.42 | 0.51 | 0.29 | 0.79 | 0.54 |
| | (3.66) | (5.37) | (4.52) | (3.19) | (2.47) | (1.83) | (2.61) | (2.20) | (2.40) | (1.52) | (3.28) | (2.40) |
| | SEm (±) | **CD (P=0.05)** | | SEm (±) | **CD (P=0.05)** | | SEm (±) | **CD (P=0.05)** | | SEm (±) | **CD (P=0.05)** | |
| A | 0.59(0.98) | **NS** | | 0.63(1.38) | **NS** | | 0.13(0.57) | **NS** | | 0.11(0.50) | **0.31(1.44)** | |
| B | 1.25(2.09) | **NS** | | 0.84(1.95) | **NS** | | 0.27(1.20) | **0.77(3.46)** | | 0.23(1.07) | **0.66(3.06)** | |
| A*B | 1.77(2.95) | **NS** | | 0.21(0.65) | **NS** | | 0.38(1.72) | **1.09(4.90)** | | 0.32(1.51) | **0.95(4.33)** | |

**Data in parenthesis are angular transformed values; NS- Non Significant*

## Table 3. Grain yield and cost:benefit ratio in PK 1029 and PK 416 cultivars of soybean

| Treatments (B) | PK 1029 (A) | | | | | | PK 416 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Grain yield (q/ha)** | **Additional grain yield (q/ha)** | **Cost of additional yield (Rs/ha)** | **Total cost of treatments (Rs)** | **Net profits (Rs)** | **Cost benefit ratio** | **Grain yield (q/ha)** | **Additional grain yield (q/ha)** | **Cost of additional yield (Rs/ha)** | **Total cost of treatments (Rs0** | **Net profits (Rs)** | **Cost benefit ratio** |
| *B. bassiana* | 25.83 | 7.27 | 7270 | 1998 | 5272 | 1:2.64 | 19.38 | 5.34 | 5340 | 1998 | 3342 | 1:1.67 |
| Diflubenzuron | 26.53 | 7.97 | 7970 | 2870 | 5100 | 1:1.77 | 22.42 | 8.18 | 8180 | 2870 | 5310 | 1:1.85 |
| NSKE 4% | 23.60 | 5.04 | 5040 | 790 | 4250 | 1:5.38 | 23.33 | 9.09 | 9090 | 790 | 8300 | 1:10.51 |
| NSKE 6% | 23.89 | 5.33 | 5330 | 1030 | 4300 | 1:4.17 | 20.83 | 6.59 | 6590 | 1030 | 5560 | 1:5.40 |
| Cow urine 20% | 22.49 | 3.93 | 3930 | 310 | 3620 | 1:11.68 | 22.15 | 7.91 | 7910 | 310 | 7600 | 1:24.52 |
| Cow urine 10% | 24.37 | 5.81 | 5810 | 310 | 5500 | 1:17.74 | 24.30 | 10.06 | 10060 | 310 | 9750 | 1:31.45 |
| Cow dung ash | 22.83 | 4.27 | 4270 | 310 | 0 | 1:12.77 | 23.33 | 9.09 | 9090 | 310 | 8780 | 1:28.32 |
| ***Triazophos 40EC*** | 28.95 | 10.39 | 10390 | 1270 | 9120 | 1:7.18 | 25.06 | 10.82 | 10820 | 1270 | 9550 | 1:7.52 |
| Control | 18.56 | - | - | - | - | - | 14.24 | - | - | - | - | |
| Mean | 24.11 | - | - | - | - | - | 21.60 | - | - | - | - | |
| | SEm ± | **C.D (P= 0.05)** | | | | | | | | | | |
| A | 0.63 | **1.81** | | | | | | | | | | |
| B | 1.34 | **3.85** | | | | | | | | | | |
| A x B | 1.89 | **5.44** | | | | | | | | | | |

** Price of soybean- Rs. 1000/quintal*

and 1: 28.32 in PK 1029 and PK 416, respectively. Raj Kumar (2000) obtained highest cost-benefit ratio 1:21.68 in one spot treatment of triazophos. However, two sprays of NSKWE gave highest net profit.

The results of the present investigation suggest that Neem seed kernel extract and cow urine were significantly effective to limit the population of stem fly. Chemical insecticide triazophos was more effective than bio-rational insecticides in the management of girdle beetle. The bio-rational pesticides were not only effective in enhancing yield but were also cheaper and safer to environment, non-target organisms and beneficial insects.

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# SOYBEAN RESEARCH

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- They should not exceed more than 1300 words (total 5 typed pages, which deal with (i) research results that are complete but do not warrant comprehensive treatment, (ii) description of new material or improved techniques or equipment, with supporting data and (iii) a part of thesis or study. Such notes require no heading of sections. It should include key words. Figures and tables should be kept to a minimum.

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Authors with in-depth knowledge of the subject are welcome to submit review articles. It is expected that such articles should consist of a critical synthesis of work done in a field of research both in India and/or abroad, and should not merely be a compilation.

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Authors should correct the proof very critically by ink in the margin. All queries marked in the article should be answered. Proofs are supplied for a check-up of the correctness of the type settings and facts. Excessive alterations will be charged from the author, Proof must be returned immediately to shorten the reproduction time.

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Ph.: 0731-2478414; 236 4879; FAX: 2470520

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