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Research News

Genome editing without the hassles of tissue culture: Hope for editing plants recalcitrant to *in vitro* manipulations

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ABSTRACT

Genome editing has become a breakthrough technology this decade to precisely modify the genome in short time. This technology has paved way for manipulating the traits of agronomic importance with ease in many crop plants. Even though plant transformation is necessary in the first step, the final product is non-transgenic; thus, attractive for social acceptance. Lack of robust transformation procedures is a limitation to use the genome editing in some crop plants. It becomes even harder when the frequency of precise editing effected by the reagents is low. Recent reports show that genome editing can be performed bypassing the tissue culture procedures; therefore, offer hope for its use in crops that are unresponsive. We briefly discuss these breakthrough techniques here.

Keywords: Biotic/abiotic stress, CRISPR/Cas9, Genome editing, Mobile sgRNA, Oilseed crops, Plant tissue culture

Conventional breeding methods that have been successfully employed so far in generating elite oilseed crop varieties are not only tedious and time taking but also are dependent on the unique combination of genes resulting from random recombination and segregation events of meiosis. In case there are agronomically superior genotypes lacking one or two traits, improving them precisely for just the target traits through recombination breeding approaches (the backcrossing method) is quite daunting as the changes brought about are not precise, often needs a number of cycles of backcrossing, and suffers from linkage drag. Hence, there is a hunt for simple and precise breeding methods that can specifically alter a genotype for only one or two traits. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated system (CRISPR/Cas) genome editing technology has come as a boon in this regard (Zhang *et al.*, 2020). CRISPR/Cas, a microbial adaptive immune system, has been established as a powerful tool to make precise genome modifications in plants. Explaining this technology briefly, Cas is an RNA-guided endonuclease that specifically targets and cleaves target DNA after recognizing protospacer adjacent motif (PAM) sequence present in close sequence context of the region of homology between guide RNA and the target sequence. The resultant cleaved DNA is repaired by two known *in-vivo* mechanisms i.e., non-homologous end-joining (NHEJ) which is error-prone and homology-directed repair (HDR) which is expected to precise but rare and less efficient. The NHEJ lead to the random nucleotide base insertions or deletions (InDels) at cleavage site which usually result in the formation of nonfunctional protein. CRISPR/Cas has revolutionized the pace of plant biology research (Manghwar et al., 2019) and made plant genome editing a reality (Zhu *et al.*, 2020). Thus, it has become as attractive and competitive field within a span of 8 years (first editing reported in 2012, Jinek *et al.*, 2012). It is noteworthy to mention here that the pioneer work by Emanuelle Carpentier and Jennifer Doudna, who sitting across the Atlantic did their basic work in establishing that CRISPR/Cas9 had evolved as a defense mechanism in bacterial system to fight the viral invasion, could be used for precise genome editing, earned them the Nobel prize in chemistry in 2020.

CRISPR/Cas9 (a Cas gene taken usually from *Streptococcus pyo-genes* has been adopted successfully in many crops for improving the traits of agronomic importance including disease resistance, quality improvement, plant architecture modification, reducing the duration, etc (Zhu *et al*., 2020). Even though this technology has made inroads into crop improvement programmes including that of oilseed crops, the limitation in adopting this technology routinely across crop plants is its dependence on protocols for obtaining the transgenic plants carrying required components (Cas9 protein and the single guide RNA) that bring about genome editing in the plant.

Delivering the machinery needed for genome editing, such as the guide RNA and Cas9, in the form of expressing cassettes into the plant cell is a critical step in realizing genome edited plants. Thus, plant transformation is a crucial and necessary step in adopting genome editing technology in any crop. Currently, two methods are widely used for DNA transfer in plants i.e., Agrobacterium-mediated and particle bombardment mediated. The former mode is often chosen owing to its simplicity and reliable transgene expression. However, in the first place, this approach demands the availability of an in vitro regeneration and transformation protocol. Besides, Agrobacterium-mediated plant genome alterations are labor-intensive, time consuming and costlier since this method is associated with aseptic *in-vitro* plant tissue culture (PTC). *In-vitro* genetic transformation and regeneration of plantlets are the major bottlenecks in PTC in

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many crop species (Altpeter *et al*., 2016). In fact, in most of the oilseed crops, high frequency transformation protocols are not available. Bypassing the PTC will certainly enhance the applications of plant genome editing technologies. Hence, innovating novel and alternative approaches that skip PTC are the need of the hour if genome editing has to become a reality on many crops including oilseed crops.

Overcoming tissue culture bottleneck: Several attempts have been made to overcome the tissue culture step for realizing transgenic plants - *in planta* transformation was a method that could successfully be employed in many crops. Floral-dip is the major *in-planta* transformation method known to date that skip PTC (Clough *et al*., 1998). This method has been followed in almost all the basic molecular biology labs worldwide. Nevertheless, this method is highly specific for Arabidopsis and related crucifers but not very successful in other major food and oilseed crops (Altpeter *et al*., 2016). Recently, two breakthrough techniques (Mahar *et al*., 2020; Ellison *et al*., 2020) have been developed that show the possible ways to generate plants without PTC procedure. Considering the new avenue these two techniques have opened in the area of plant genome editing, here we provide an overview of these techniques and also narrate the prospects of adopting them to alter oilseed crops for specific target traits. The first method uses the genes that are known to be involved in induction of meristems to induce de novo shoot production on *in situ* raised plants while the second one uses virus induced gene silencing (VIGS) vector system to transiently express the single guide RNA (sgRNA) in the transformed cells to effect genome editing.

Technique 1: De-novo induction of meristems: Molecular dissection of the development of a whole plant from the cells that are meristematic, led to understanding of the genes involved in the process. Plant developmental regulators (DRs) such as BABY BOOM (BBM), WUSCHEL (WUS), SHOOT MERISTEMLESS (STM), MONOPTEROS (MP), and ISOPENTENYL TRANSFERASE (IPT) play vital role in determination of apical meristem (Barton, 2010). This understanding led to manipulating the expression of DRs ectopically to realize increased transformation efficiency. Overexpression of the maize BABY BOOM (BBM) and WUSCHEL2 (WUS2) genes caused for high transformation frequencies in several recalcitrant maize inbred lines, sorghum immature embryos, sugarcane callus, and indica rice callus (Lowe *et al*., 2016). Ectopic expression of various combinations of these DRs induces differentiation of somatic cells into meristematic cells (Nelson-Vasilchik *et al*., 2018).

Based on this understanding, Maher *et al*. (2020) reported a first-of-its-kind plant transformation method called Fast-TrACC (Fast-Treated *Agrobacterium* CoCulture) to deliver desired plasmid DNA constructs to Nicotiana benthamiana through transient expression. They tested twelve combinations of different DRs (BBM /ipt /MPΔ /STM /Wus2 /All /BBM&ipt /BBM&Wus2 /IPT& MPΔ /STM&MPΔ /Wus2&ipt /Wus2&STM) driven with various promoters (*Zm*Ub1/*Cm*YLCV /35S /nos promoter) for successful meristem formation on in situ raised plants at the site of injury and transformation. Two combinations i.e., Wus2/STM and Wus2/ipt produced the transgenic meristems at high frequency. Encouraged with this result, they combined this observation along with genome editing process to obtain genome editing in the de-novo forming shoot meristems. To achieve this, Maher *et al*. (2020) used a technique called Agroinfection wherein the viral replicons could be introduced into the cells through Agrobacterium mediated procedure. *Agrobacterium* harboring desired viral replicons carrying expression cassettes of DRs, luciferase marker and guide RNA which targets two phytoene desaturase (PDS) homologs, was infiltrated at cut shoot apices of Cas9-expressing soil grown *N. benthamiana* plants. The premise was that DRs would increase the frequency of meristematic cells at the site of infiltration increasing the chance of transformation, and the gRNA would edit the PDS gene by using the Cas9 protein in the plant (encoded by the *cas9* transgene in the plant) leading to cells devoid of chlorophyll and thus enabling easy identification of transformed (genome edited) cells. This logic and rationale worked leading to a whopping 30% success rate of getting genome edited white shoots at the site of Agroinfection. This procedure is illustrated in Figure 1A. Thus, Maher *et al*. (2020) successfully bypassed the time-consuming PTC method and realized the genome edited tobacco plants in a short time. They extended the same protocol to other agronomically important crops like tomato, potato and grape and obtained similar results. Thus, this approach involving injecting of DRs and gene-editing reagents could be effectively used to create genome edited transgenic crops.

Technique 2. Editing with mobile single guide RNAs (sgRNA): Plant viruses have offered several tools (as transformation vectors) in plant biotechnology. The advantage is that they do not integrate into plant genome and do not pass through germline to the next generation. Ribonucleic acid (RNA) viruses are not much exploited as vectors in plant biotechnology except being used for viral-induced gene silencing (VIGS), a method used effectively in functional genomics studies to establish the role of selected genes. Thus, these vectors could be used for introducing transgene(s) for temporary expression and the effects caused by the introduced genetic elements are temporary. But the only limitation has been the 'carrying capacity of the cargo' (the size of the expression cassettes) of these RNA virus-based vectors. Hence, several researchers who wanted to use RNA vector based expression system for genome editing, successfully used RNA viruses such as tobacco mosaic virus-derived vector (TRBO), tobacco rattle

virus (TRV), pea early browning virus (PEBV) and barley yellow striate mosaic virus (BYSMV) to deliver sgRNAs against the target genes in plants that were already expressing Cas9 but that resulted in low frequencies of gene editing in somatic cells which was not inherited (Cody *et al*., 2017; Ali *et al*., 2018; Gao *et al*., 2019). Heritable gene editing could be possible if the sgRNA could get an access to the plant germline cells. To accomplish this, researchers took the advantage of plant endogenous mobile signals. Plant produce many mobile endogenous small RNAs i.e., FLOWERING LOCUS T (FT) RNA, methionine transfer RNA ($tRNA^{Met}$), isoleucine tRNA ($tRNA^{Ile}$), micro RNAs (miRNAs) and many other non-coding RNAs (ncRNAs). These small RNAs can systemically move throughout the plant including apical meristems and germlines (Notaguchi *et al*., 2015).

Based on this cumulative knowledge, in their ingenuous experiments Ellison *et al.* (2020) generated heritable mutations in *N. benthamiana* with remarkable efficacy. First, they generated SpCas9 expressing *N. benthamiana* plants and then infected them with *A. tumefaciens* carrying mobile sgRNA in tobacco rattle virus (TRV) vector. They effectively tested and confirmed heritable mutants with three mobile elements i.e., *FT* mRNA, $tRNA^{Met}$, and $tRNA^{Ile}$. They observed 90-100 % editing efficiency in somatic tissue that resulted in 65-100% heritable mutations. They succeeded in editing two essential genes i.e., phytoene desaturase (PDS) and AGAMOUS (AG) by multiplexing the sgRNAs against both the genes. The procedure followed has been illustrated in Figure 1B.

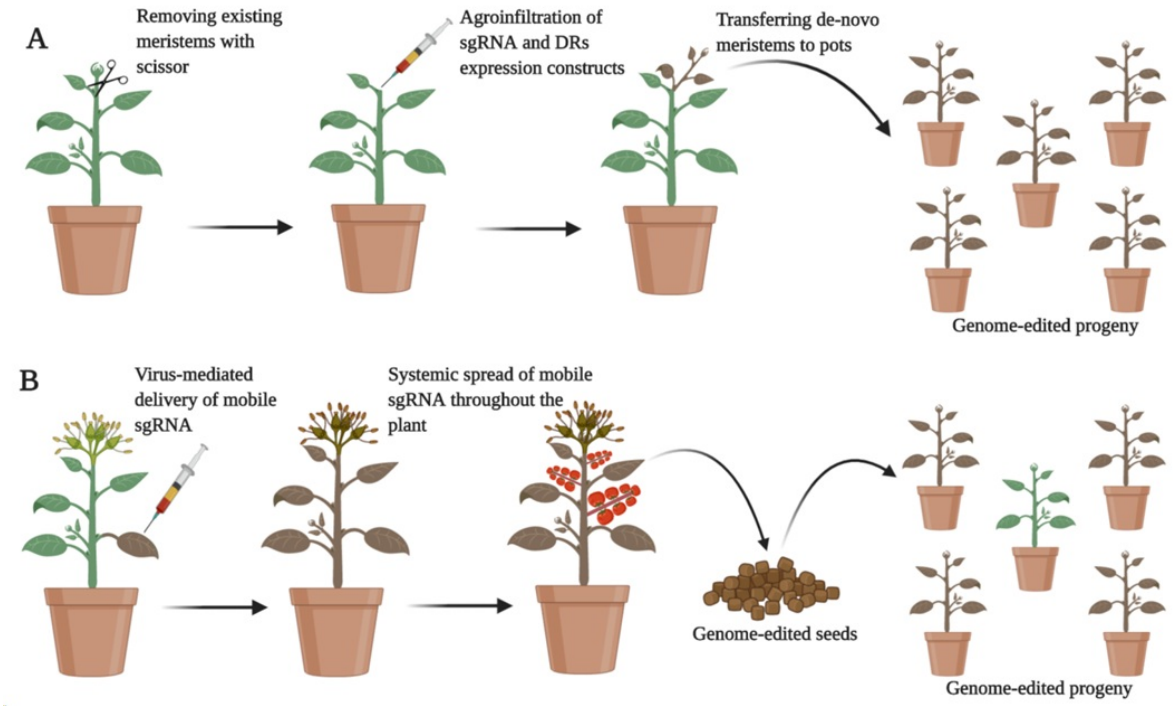

Fig. 1. Illustration of the methods for obtaining tissue culture-free genome editing in Cas9-overexpressing soil-grown plants. Genome-edited progeny shown in brown color while non-edited in green color. A) De-novo induction of meristems (method as described in Maher *et al*., 2020). The meristem is removed with scissor. The cut site is then perfused with *A. tumefaciens* cultures carrying developmental regulators (DRs), genome editing reagents (sgRNA against phytoene desaturase) and luciferase (for visual confirmation of construct delivery to plant cells). Over time, genome-edited plantlets that were chlorotic, produced from the site of delivery were transferred to the pots for next generation. B) Editing with mobile single guide RNAs (method as described in Ellison *et al.,* 2020). Viral vector comprising mobile sgRNA are infiltrated to bottom leaf by Agrobacterium infiltration. Mobile elements are known to move from leaf to shoot throughout the plant including germline cells. Seeds obtained from the infected plant screened for desired gene editing.

Caveats still to be addressed: As discussed above the two recent developments have opened up new vistas for exploiting the genome editing in crops that are difficult to regenerate through tissue culture. However, these two methods still depend on generation of constitutive *Sp*Cas9 over expressing transgenic plants which serve as the starting material and thus totally do not obviate the dependence on tissue culture. Also, in the first method (Maher *et al.*, 2020), it needs to be explored whether the developmental regulators would lead to formation of de-novo meristems in all crops and the resultant plantlets could be established in the soil to give the next generation plants. Wider application of the second technique (Ellison *et al.*, 2020) would depend on the availability of appropriate viral vectors that are effective in

the chosen crop. Between the two techniques, the second one could perhaps become more tissue culture independent if Cas9 variants that are smaller in size are developed/ identified so that and they could also be cloned within the RNA virus-based vector along with sgRNA cassette and thus the tissue culture step could be totally avoided.

There are other caveats also for application of CRISPR/Cas9 technique for precise editing of the genes of interest. These include, the requirement of PAM specificity around the region targeted for editing, random off-target mutations, selection of sgRNA, balanced in-vivo expression of sgRNA and Cas cassettes in the plant for high editing efficiency, availability of whole genome sequence of the crop for the selection of effective sgRNA through online web-tools so that homologs, if any, are not targeted for editing, and identification and optimization of suitable crop specific promoters for effective expression of Cas and sgRNA. Lower frequency of genome editing is yet another important criterion that necessitates production of a larger number of transgenic plants to achieve the manifestation of the traits as required.

Prospects of genome editing in oilseed crops: Breeding objectives in oilseed improvement programmes, besides increasing the seed and oil yield, include imparting biotic and abiotic stresses, reducing the seed losses due to shattering, improving the quality of oil produced, biofortification, reducing the duration, etc. Genome editing technology has already proven useful in tackling similar issues in other crops and thus could be an important technique in improving oil yielding crops as well. In plants, synthesis and accumulation of oils are controlled by complex gene network. Role of these networks is mostly unknown and need to be elucidated. Finding and tweaking these genes involved in desired networking pathways for oil accumulation can be exploited to increase oil production. There are several examples of successfully employing genome editing technique in oilseed crops and readers are referred to recent reviews for more information (Subedi *et al*., 2020).

In conclusion, the two techniques discussed here would be a major step towards achieving genome editing in crops that are recalcitrant to plant tissue culture methods. Further, if the other limitations of large-scale application of CRISPR/Cas9 mediated genome editing are overcome, oilseed crops could be altered precisely to meet at least a few breeding goals more rapidly and accurately.

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Research Accounts

Experiences in development of DOR Bt-1 technology: the highs and lows during the sojourn

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Insect pest management is important to save the crop from yield losses and increase productivity. In India, about 33% of the crop loss occurs due to insect pests and is worth of about ₹ 200 thousand crores annually. In addition substantial volume of agricultural exports is rejected every year due to pesticide residues. The pesticides are unsafe for environment and human health; their misuse by farmers further aggravates the problem. Therefore, sustainable and eco-friendly pest management practices are needed. In this article, I share my personal experiences during the two-decade sojourn, marked by struggles and breakthroughs, which culminated in a successfully commercialized biocontrol technology that has reached the farmers.

Beginning of the journey: During 1980s, the concept of Integrated Pest Management (IPM) gained the momentum due to undesirable effects of chemical pesticides. The IPM programmes were aimed at minimizing the use of chemical pesticides by alternative means such as biological control, which uses the naturally occurring organisms or their by-products for management of insect pests and diseases. The bio-pesticides (microbial) are safe and eco-friendly. Due to biodegradable nature, they leave no residues on crops, and do not contaminate the environment. However, lack of characterized biological agents was a concern. At ICAR-IIOR (formerly DOR), the project on microbial control of insect pests was initiated by me in 1988, with an aim to exploit them in the IPM programmes. This work was further strengthened by the externally funded projects (ICAR Cess Fund, AP-NL Biotechnology Programme, NATP, ICAR-Network Project on AMAAS) for development of microbial insecticides involving *Bacillus thuringiensis,* entomopathogenic fungi (*Beauveria bassiana* and *Nomuraea rileyi*) and baculovirus of castor semilooper.

The microbial pesticides are host specific and do not interfere with other biotic systems. Developing a microbial insecticide involves several milestones:

- collection of soil samples and infected cadavers of insects from field
- isolation and identification of isolates
- identification of virulent / potent isolates
- characterization of isolates for their efficiency as insecticides, host range, active principles
- development of cost-effective mass production protocols
- development of pesticide formulations with extended shelf-life at ambient temperature
- determination of effective dose through laboratory evaluation
- field testing
- shelf-life determination
- development of long-term storage mechanism for isolates
- intellectual property protection
- registration
- commercialization

At the beginning, information on microbial pesticides was scanty; limited to reports of the natural occurrence of dead larvae due to infection by entomofungal pathogens and baculoviruses, and identification of virulent isolates in the laboratory studies, no formulations were available for field use, and no guidelines were available for registration in India.

Dealing with biological agents is problematic because we must ensure conducive environment for their multiplication, no contamination, quality parameters at the production level and that they are effective under varying conditions of the crop growth. During 1990s, ICAR-IIOR dealt with several major insect pests namely semilooper (*Achaea janata*) on castor, gram pod borer (*Helicoverpa armigera*) on sunflower and groundnut, and tobacco caterpillar (*Spodoptera litura*) on castor and groundnut. These insect pests are polyphagous and voracious defoliators, and managing them was a challenge.

At this juncture, I decided to work on *Bacillus thuringiensis* (Bt), a wonder bacterium that had been commercialized for use against lepidopteron pests in crops.

Initiation of work on *Bacillus thuringiensis*: The Bt is a ubiquitous soil bacterium. It was first isolated in 1901 by a Japanese biologist Shigetane Ishiwata from a diseased silkworm. It was rediscovered 10 years later by Ernst Berliner in Thuringen, Germany, in a diseased caterpillar of flour moth. It was classified in 1911 as type species *Bacillus thuringiensis* and remains the most widely used biocontrol agent to date. The Bt was used as a biological insecticide in the early 1920s in France; Sporeine - the first commercial Bt product became available in France in 1938. In the USA, the Bt was widely used during 1950s. However, research and development on Bt progressed at slow pace after 1950

because of the widespread adoption of cheaper but highly toxic synthetic chemical insecticides. In 1956, the Pacific Yeast Product Company developed an industrial process known as submerged fermentation, which allowed production of the Bt on a large scale. Subsequently, new products of the Bt were developed and applied; especially in niche markets where petroleum-based chemicals were not registered or ineffective, or costly.

Production of the Bt is dominated by multinational companies as it requires high capital investment. High cost of the commercial Bt formulations (₹ 1200-2000/kg during 1990s) and lack of availability limited the use of Bt in India. So, I thought 'my Bt products' would fill the gap of its availability in India. Except for testing some pre-registration imported products of Bt supplied for testing against insect pests of oilseed crops I had no experience of working on new Bt isolates. However, tests with commercial imported products had revealed the efficacy of Bt against castor semilooper *i.e.,* capacity to cause immediate feeding cessation and result in larval mortality within 2-3 days (Vimala Devi *et al*., 1996; Vimala Devi and Prasad, 1999).

Development of DOR Bt technology: I procured several strains of various Bt subspecies from the Bacillus Genetic Stock Center (BGSC), Ohio university, USA to use them for starting research work as well as using them as reference material for later studies with indigenous isolates. We collected soil samples and dead larvae of semilooper in 1989 from the castor fields in Mahbubnagar and Nalgonda districts (Telangana). The fields had no history of Bt use. Using a process for selective isolation of Bt (Travers *et al*., 1987), we had a collection of over 100 isolates.

Then, we looked for a 'magic isolate' which would kill the target insects at the lowest dose, through screening against castor semilooper larvae in laboratory bioassays. Luckily, one local isolate, DOR Bt-1, belonging to *Bacillus thuringiensis* var. *kurstaki* isolated from a dead castor semilooper larva collected from the farmers' fields at Kothakota (Telangana) was identified as a really potent isolate (Vimala Devi *et al*., 2001).

Major bottleneck - cost effective production of Bt cracked: Development of low-cost mass production protocol is the key for commercial success. At the time of initiation of this project, no information was available in the public domain for isolation, identification, and mass multiplication of Bt. The multinational companies like Abbott were producing commercial Bt products through submerged fermentation using huge fermenters (1000-2000 litres capacity) but the information was not publicly available. An 11-litre fermentor was provided in the project (costed 17 lakh rupees in 1988) to multiply Bt in the fermentor and validate the efficacy of the formulation at field level so that fermentors could be established at village level to undertake Bt production. I had a training on submerged fermentation at IMTECH, Chandigarh for 10 days before initiating work with fermentor. We succeeded in developing the fermentation technology for production of DOR Bt 1 but we did not transfer it to the villages because it was impractical in terms of expertise and cost. This became a bottleneck and a stumbling block for furthering the project objectives.

As Bt is an aerobic bacterium with specific growth requirements of pH 7.5 and temperature 30°C, solid state fermentation (SSF) was never considered an option for Bt mass production. Despite this, we started exploring the possibility of employing SSF for Bt mass production using DOR Bt-1.Elsewhere, mass production of Bt on the principle of solid-state fermentation (SSF) had been successfully carried out on a bench scale using media ingredients that were easily available and inexpensive (Fernandez-Larrea Vega, 1999; Moraes *et al*., 1998; Morris *et al*., 1996).

We had to make hundreds of permutations and combinations of media employing locally available cheap substrates including agricultural byproducts/wastes like wheat bran, rice bran, molasses, soybean etc. and undertook multiplication in plastic tubs covered with polythene sheets. Maintaining a continuous supply of air was a challenge. Intermittent aerations at 24 h intervals were required to be given in the laminar airflow. After a great deal of experiments and without mentioning about all the failed trials and struggles, after almost 2-3 years of concerted efforts, we succeeded in developing a simple, low-cost mass production protocol on the principle of SSF. This was the first report of Bt multiplication through SSF. These initial studies for multiplication through SSF were published in the Journal of Invertebrate Pathology in 2005 (Vimala Devi *et al*., 2005). This invention was brought the Bt production within the reach of small and medium entrepreneurs. The material cost of production was around Rs.30 per kg (Vimala Devi *et al*., 2005). Considering the novelty and utility of the methodology, a patent application was filed for the process in 2002 (732/Del/2002). Later, we improvised the protocol to enable mass multiplication in polypropylene covers. We used locally available sponges of fine pore size for enabling continuous aeration. This helped us to overcome the problem of aeration and reduced the chances of contamination (Vimala Devi *et al*., 2020).

Briefly, the other features of the developed methodology include:

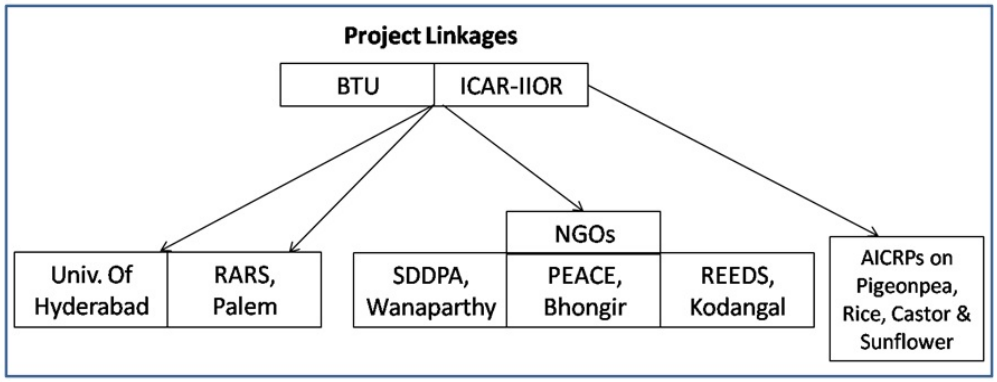
- The production is simple, less demanding in technical skill and capital investment, eliminates the need for a fermentor
- It has the potential to enable large-scale localized production of Bt through establishment of cottage industry/ micro-enterprises

The developed formulation of Bt had the following characteristics:

- It was effective against castor semilooper, leaf folder in rice and pod borer in pigeon pea
- It caused immediate feeding cessation and death within 2-3 days in the target insect pests
- It was safe to natural enemies of the pest, non-target organisms and the target crop
- It was biodegradable and more importantly safe to human beings and the ecosystem
- It had long shelf-life (2 years minimum) at room temperature

Once the mass production technology was established, the next step was to validate the Bt product in the fields as well as to scale it up and work out economics of scaling. This needed transfer of the technology to potential entrepreneurs and producers including the farmer self-help groups and validate the technology on farmers' fields. This needed multiple players and a close knit interaction among them. Therefore, establishing linkages among all the different stake holders in the process of taking technology to commercial scale was a crucial intervention needed. Thus, our next task was to establish linkages among entrepreneurs as well as farmers.

Linkages for technology development and transfer: Several strategic organizational linkages were created in the project for effective technology development and successful transfer to the farmers. It was a typical case of partnership between funding agency, research institutions, NGOs and farmers as indicated in the scheme below:



Briefly, the following actions were undertaken by different groups:

- Technology development was undertaken by scientists at DOR in association with University of Hyderabad, RARS-Palem and 3 NGOs, SDDPA, PEACE and REEDS. Scientists from entomology, biotechnology and insect physiology disciplines carried out the basic research aspect of the technology development. Technology validation at field level was done by farmers of Mahbubnagar and Nalgonda districts duly facilitated by the local NGOs.
- Awareness was created among the farmers at the village level through 20 training programmes from 2000-2004 about the use and efficacy of Bt against castor semilooper, its mode of action and safety to the natural enemies *viz.,* parasitoids and predators. Such an overwhelming and enthusiastic response from the farmers, gave that feeling of fulfilment and satisfaction.
- The farmers were also trained in field schools to identify the various stages of castor semilooper right from egg to the late larval instars as well as the various parasitoids. Emphasis was laid on undertaking the Bt sprayings when the semilooper larvae were in the early stages.
- Demonstrations of DOR Bt-1 formulation were conducted on castor in more than 200 acres in farmers' fields during 2001-04. Each farmer was provided with the Bt formulation for an area of 2 acres and another acre was maintained as per his practice for pest management. Thus, farmers were made to observe the differences in terms of natural enemies, frequency of sprays required, yield, etc between the Bt sprayed fields and quinalphos sprayed fields (Vimala Devi and Sudhakar, 2006).
- Two microenterprises were established in association with NGOs - one with Society for Development of Drought Prone Area (SDDPA) and second with Grameena Mahila Mandali (GMM) at Mahbubnagar and Nalgonda districts, respectively. Around 15 high school dropout boys and girls were trained in Bt production and the formulation was supplied to farmers for use in the field for pest management. Farmers purchased Bt from the micro-enterprises @ ₹ 250-300 per kg while the cost

of commercial Bt formulations ranged ₹ 1200-2000 per kg. Thus, Bt was brought within the reach of the resource-poor farmers of the two dryland districts at an affordable price. Thus, my dream that my research efforts should fructify in giving product to resource poor farmers came true (Vimala Devi and Rao, 2005 a, b, c).

- A production unit was also established by us in the Rural Technology Park (RTP) of NIRD, Hyderabad in association with SDDPA at the invitation of the then Director General and showcased as a rural technology. Farmers from several states purchased the Bt formulation from this unit and used it in their fields.
- The technology application was later extended to management of gram pod borer in pigeon pea.
- Molecular characterization of DOR Bt-1was carried out using cry gene specific primers and Rep-PCR for demonstrating uniqueness of the isolate (Kumar *et al*., 2003; Reddy *et al*., 2012 a, b).
- Validation of the efficacy of the formulation was carried out through multi-location testing under the All India Co-ordinated Research Projects (AICRPs) on castor and pigeon pea.

Intellectual property rights (IPR): Intellectual property (IP) refers to creations of the mind, such as inventions; literary and artistic works; designs; and symbols, names and images used in commerce. Intellectual Property Rights (IP Rights) are like any other property rights which are intangible in nature. The IP Rights usually give the creator an exclusive right over the use of his/her creation for a certain period. It was for the first time that a protocol was developed for Bt production through SSF. Since DOR Bt-1 technology had a commercial value, IP protection became utmost important. At that time, there was no mechanism/system in place for filing of patent applications in ICAR. We explored the options available and corresponded with ICAR in this regard. Finally, a provisional patent application for the process was filed by ICAR in 2002 and the complete patent application in 2003.

Registration with Central Insecticides Board (CIB): The import, manufacture, sale, transport, distribution and use of microbial pesticides are regulated in India under the Insecticides Act, 1968 and rules framed there under. The Registration Committee of CIB grants registrations, after scrutinizing and verifying claims with respect to their bio-efficacy and safety to human beings and animals. Registration of bio-pesticides in India was approved around the year 1998. Bio-pesticides of botanical and microbial origin were included in the schedule of the Insecticide Act 1968. To obtain registration, data had to be generated on mammalian toxicity and eco-toxicity (from referral labs), bio-efficacy - lab and field (from ICAR institutes /SAUs), safety to natural enemies, phytotoxicity, shelf-life, container content compatibility, packaging, and labelling. Guidelines are available at http://ppqs.gov.in/divisions/cib-rc/guidelines?page=2. So the registration process was not an easy task - it needed the data more than what we required for proving its efficacy. Thus, the journey of developing a commercial product was far from over. As per the requirement, we started generating the relevant data for DOR Bt-1 formulation. The formulation was registered with the CIB in 2005 under the trade name KNOCK.W.P. [Registration no. CIR-511/2005(256)] under section 9(3b) with the Central Insecticides Board, Govt. of India. It is the first formulation of a microbial pesticide from the ICAR and from any public sector registered for commercial use.

Under the APNL project, micro-enterprises were established for enabling localized production and sale of the DOR Bt-1 W.P. formulation to the farmers in Mahabubnagar and Nalgonda districts which made the registration of the formulation essential. Several local unemployed youth were trained to undertake Bt production in the micro-enterprises. This initiative helped in spread of the technology among the local farmers.

Commercialization: The National Agricultural Policy 2001 laid special emphasis on IPM with emphasis on the use of "bio-agents in order to minimize the indiscriminate and injudicious use of chemical pesticides". However, one of the major constraints faced in adopting the IPM practices was the non-availability of good quality biotic agents at the farm level. Biopesticides production in India was being promoted commercially by medium range entrepreneurs. The DOR *Bt1* production could not be undertaken by them due to high capital investment for production as well as the difficulty in generating data for registration. The sole purpose of technology development under the project was to bring the Bt technology in reach of the resource poor farmers of Mahbubnagar and Nalgonda districts. It was never meant for the purpose of commercialization. Data generation for registration was expensive. Hence, data generation by the IIOR for the purpose of registration encouraged several medium range firms to approach IIOR and request for licensing of the technology during the year 2003. No guidelines existed in ICAR for commercialization of the technologies. Subsequently, with the approval from ICAR, IIOR started licensing of the DOR Bt 1 to the bio-pesticide industry in India from July 1, 2006 on a non-exclusive basis. The technology package included DOR Bt-1 strain along with data for provisional registration and training in the production technology through solid state fermentation. The technology has been so far licensed to 40 bio-pesticide firms in India and earned a revenue more than one crore INR. The technology has been popularized among farming community by publishing easily readable pamphlets as well as by releasing the documentary in English as well as vernacular language (listed at the end of this article).

This initiative enabled the licensee firms to seek registration of the formulation with the Central Insecticides Board and manufacturing license from respective state Governments to undertake commercial production of the DOR Bt 1. Majority of the firms received the provisional registration and sold their Bt formulations under different trade names *viz*., Cezar, Caterpillin, JAS BT, VBT, R.B. Bt, Prasar, Dipole, Beater etc. Thus, DOR Bt 1 technology successfully reached the farmers. Thus, it took around 15 years to develop a technology and make it accessible to the farmers through commercialization. The project became a case study for ICAR with respect to IPR issues (Hegde and Vimala Devi, 2004) and technology commercialization and has been included in the IP guidelines of ICAR.

In accordance with the Biodiversity Act, 2002 and Rules 2014, IIOR, Hyderabad shared 3% of the licensing fee with Biodiversity Management Committee (BMC) at Kothakota through the Telangana State Biodiversity Board (TSBB). The BMC was constituted by TSBB at Kothakota, Mahbubnagar district since the DOR Bt-1 isolate was obtained from that village. The BMC has the responsibility of using these funds for betterment of the village including

creation of awareness on conservation of biodiversity. Since DOR was the first institute to set an example in access and benefit sharing by sharing its license fee with the BMC, it received the prestigious UNDP award as runner up under the category "Successful Mechanisms/Models for Access and Benefit Sharing" at the "India Biodiversity Awards 2016".

DOR Bt-1 formulation sold under different trade names

Novelty in technology development and outreach to farmers, intellectual property protection initiatives, commercialization of the technology for large scale promotion in India through public-private partnership (PPP) was unique to the project. My efforts in this regard were recognized by ICAR and I was awarded the "Panjabrao Deshmukh Outstanding Woman Scientist Award" for the year 2017. The Society for Biocontrol Advancement in India has also recognized my efforts by selecting me for the "Dr. S.P. Singh Memorial Lifetime Achievement Award" for 2019.

So what started as a passionate dream to do something to the resource poor oilseed farmers culminated in a product that had many firsts to its credit. It taught me that perseverance is the most important ingredient for success. At the end, it has become an example to many who want to start a long journey with a single mission and conveys that if one is ready to take on the challenges and diligently work towards solving each one of the hurdles, it leads to successful completion of the mission. So, success does not just come on a platter but it needs to be carved out with well planned experimentation.

Conclusion: Resurgence in academic and industrial research for biopesticide development occurred in response to the problems associated with the use of synthetic chemicals. As a result, the development of new biopesticides continued to increase since the mid-1990s. Increased biopesticide adoption has resulted, in part due to rapid expansion of organic agriculture during the past decade. Thus, DOR Bt technology was one of the first, indigenous technology for eco-friendly pest management. It was a trend-setter for IPR protection as well as technology licensing in ICAR, enabled indigenous Bt production and brought the formulation within the reach of the farmers at an affordable price, currently being sold ₹500-1000 per kg in contrast to the other Bt formulations sold by multinational companies at ₹2000-4500 per kg. We also broke the myth that Bt cannot be multiplied through SSF. The DOR Bt -1 technology development is a perfect example of basic, strategic, and applied research through a bottom-up approach involving multi-disciplinary and multi-institutional collaboration and outreach in a public private partnership (PPP) mode.

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Sequential application of diclosulam and cycloxydim for control of mixed weed flora in *rabi*-summer groundnut (*Arachis hypogaea* L.)

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ABSTRACT

A field experiment was conducted during winter season (*rabi*-summer) of 2018-19 at S.V. Agricultural College Campus of Acharya N. G. Ranga Agricultural University, Andhra Pradesh, to evaluate the effect of pre-emergence (pendimethalin 38.7% CS, pendimethalin 30% EC and diclosulam 84% WDG @ 725, 1000 and 20 g/ha, respectively) and post-emergence (haloxyfop-p-ethyl 10.5% EC @ 135 g/ha and cycloxydim 20% EC @ 100 g/ha) herbicides on weed growth and yield of groundnut. The pre-dominant weed flora associated with groundnut was *Cyperus rotundus* L. (42%), *Boerhavia erecta* L. (15%), *Dactyloctenium aegyptium* (L.) Willd. (11%) and *Commelina benghalensis* L. (9%). Pre-emergence application of diclosulam 20 g/ha supplemented with hand weeding (HW) at 40 DAS or cycloxydim 100 g/ha at 20 DAS resulted in lower density and dry weight of all the categories of weeds including *Cyperus rotundus*. Pre-emergence application of diclosulam 20 g/ha resulted in phytotoxicity rating of "1" in 0-10 scale with yellowing of young leaves in groundnut and the crop recovered within 25 days after its application. Pre-emergence application of diclosulam 20 g/ha supplemented with HW at 40 DAS resulted in higher yield components and pod yield of groundnut, which was comparable with sequential application of diclosulam 20 g/ha as pre-emergence and cycloxydim 100 g/ha at 20 DAS. However, the former weed management practice obtained higher net returns and benefit-cost ratio than latter. These two weed management practices increased the pod yield of groundnut tby 48.8 and 44.2 %, respectively compared to un-weeded check.

Keywords: Cycloxydim, Diclosulam, Haloxyfop-p-ethyl, Groundnut, Pod yield

Groundnut (*Arachis hypogaea* L.) alone contributes about 40% of the total oilseeds production and 45% of the total area in the country. It is cultivated over an area of 4.89 million hectares with a production of 8.92 million tonnes and an average productivity of 1825 kg/ha in India during 2017-18. In Andhra Pradesh, it is grown in an area of 8.19 lakh hectares with annual production of 10.68 lakh tonnes and an average productivity of 1304 kg/ha during 2017-18. The productivity is very low as compared to USA and China mainly because, the crop is mostly grown as rainfed conditions in drylands, where low fertility and low input management often subjected to the vagaries of the weather conditions (Ajay *et al.*, 2018; Mallic *et al.*, 2018). *Rabi*-summer groundnut occupies an area of 0.78 lakh hectares in Andhra Pradesh, but productivity is low due to increased pests and diseases as well as heavy weed infestation. There is an urgent need to explore the possibilities for enhancing productivity of groundnut to increase the production of this important oilseed crop (Walia *et al.*, 2007).

Groundnut is highly susceptible to weed infestation because of its slow growth at initial stages (upto 40 DAS) and short stature with underground pod bearing habit. Weed flora associated with groundnut compriseddiverse species reduce the pod yield up to 89 % (Jat *et al.*, 2011). Unlike other crops, weeds interfere with pegging, pod development and harvesting of groundnut, besides competing for essential growth resources. Hand weeding and use of intercultural operations reduce the weed population considerably, but the timing and frequency is critical for effective weed control. Pendimethalin is the commonly used dinitroaniline herbicide for control of annual grasses and some of the small seeded broad-leaved weeds, but less effective against perennial sedge i.e. *Cyperus rotundus* and some of the broad-leaved weeds like *Commelina benghalensis* L and *Trichodesma indicum* L. (Sivasankar and Subramanyam, 2011), which are predominant weeds associated with *rabi*-summer groundnut in Andhra Pradesh. Post-emergence application of imazethapyr 75 g/ha is recommended for control of weeds in groundnut presently, but the choice of succeeding crops is limited because imazethapyr persists in soil and plant for longer time with a half-life period of 33 months and is not effective against grasses (Sondhia *et al.*, 2015). Further, pre-emergence herbicides have proved remarkably effective against weeds up to 20-25 DAS, but late emerging weeds interfere with pegging, pod development and harvesting.

Use of pre and post-emergence herbicides offers an alternative viable option for effective and timely control of weeds in groundnut, but each herbicide has its own spectrum of weed control. Pre-emergence application of pendimethalin 1 kg/ha + post-emergence application of quizalofop ethyl 50 g/ha at 20 DAS was most effective in controlling weeds in groundnut during *kharif* season (Vaghasia *et al.*, 2014). In recent years, new generation low dose and high efficiency

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herbicide molecules such as diclosulam, cycloxydim and haloxyfop-P-ethyl are available in soybean, which were found to exhibit broad-spectrum weed control with low mammalian toxicity compared to high volume herbicides like pendimethalin. In this context, there is a need to evaluate these low dose and high efficiency herbicides for control of weeds including perennial sedge, *Cyperus rotundus* and *Commelina benghalesis* in *rabi*-summer groundnut. Keeping this in view, the study was undertaken to find out the suitable pre-emergence and post-emergence broad-spectrum herbicide for management of weeds in *rabi*-summer groundnut.

MATERIALS AND METHODS

A field experiment was conducted during the winter season (*rabi*-summer) of 2018-19 at S.V. Agricultural College, Tirupati campus of Acharya N.G. Ranga Agricultural University, Andhra Pradesh, India, which is geographically situated at 13.5°N latitude and 79.5°E longitude and at an altitude of 182.9 m above the mean sea level in the Southern Agro-climatic Zone of Andhra Pradesh. The soil was sandy loam in texture having 0.4% organic carbon, soil pH 6.80, 216 kg available N, 23 kg available P and 196 kg available K/ha. The eleven treatments consisted of pre-emergence application of pendimethalin 38.7% CS @ 725 g/ha and diclosulam 84% WDG @ 20 g/ha alone and supplemented with hand weeding (HW) at 40 days after sowing (DAS) or post-emergence application of haloxyfop-p-ethyl 10.5% EC @ 135 g/ha or cycloxydim 20% EC @ 100 g/ha at 20 DAS including pre-emergence application of pendimethalin 30% EC @ 1000 g/ha, two HW at 20 and 40 DAS and un-weeded check which were laid out in a randomized block design with three replications. Groundnut cultivar 'Kadiri-6' was sown on 13 December, 2018 at a spacing of 22.5 cm x 10 cm. Totally, eight irrigations were given each at 5 cm depth during crop growth period. Pre-emergence herbicides were applied at 1 DAS and post-emergence herbicides were applied at 20 DAS by using power operated knapsack sprayer fitted with flat-fan nozzle with spray fluid of 500 L/ha. The crop was supplied with recommended fertilizer dose of 20 kg N, 40 kg P_2O_5 and 50 kg K_2O/ha through urea, single super phosphate and muriate of potash, respectively to all the plots as basal. Top dressing of 10 kg of N in the form of urea was applied at 25 DAS. Data on weeds were recorded at harvest in each plot with the help of quadrant measuring 50 x 50 cm. Weed samples were sun dried at 70°C until constant weight was attained. Weed control efficiency of each treatment was calculated (Mani *et al.*, 1973). Density and dry weight of weeds were transformed to square root transformation ($\sqrt{X+0.5}$) to normalize their distribution. The phytotoxicity rating of pre-emergence and post-emergence herbicides were observed at 10^{th} and 5^{th} day after application of herbicides, respectively on a 0-10 scale (Singh and Rao, 1976). All data were analysed using ANOVA and the least significant difference values at 5% level of significance were calculated to find out the differences between treatment means.

RESULTS AND DISCUSSION

Weed growth: The predominant weed species observed in un-weeded check plots at harvest were *Cyperus rotundus* L. (42%), *Boerhavia erecta* L. (15%), *Dactyloctenium aegyptium* (L.) Willd. (11 %), *Commelina benghalensis* L. (10%), *Digitaria sanguinalis* (L.) Scop (8%), *Cleome viscosa* L. (6%), *Phyllanthus niruri* L. (4%) and others (4%). All the weed management practices significantly influenced the weed growth of groundnut (Table 1). The lowest density and dry weight of total weeds including grasses, sedges and broad-leaved weeds was recorded with pre-emergence application of diclosulam 20 g/ha supplemented with HW at 40 DAS, which was significantly superior than the rest of treatments with respect to weed density and it was comparable with pre-emergence application of diclosulam 20 g/ha fb cycloxydim 100 g/ha applied at 20 DAS, with respect to weed dry weight. Both the weed management practices were significantly superior in reducing density and dry weight of total weeds than rest of the weed management practices.

Pre-emergence application of diclosulam 20 g/ha was found to be very effective in controlling all the categories of weeds including predominant perennial sedge, *Cyperus rotundus* compared to pre-emergence application of both the formulations (CS and EC) of pendimethalin. This might be due to the extended herbicidal activity of diclosulam against weeds up to harvest because of higher half-life period and better leaching behaviour compared to pendimethalin, which might have increased the concentration of soluble diclosulam at weed seed zone. Tomlin (2000) also concluded that diclosulam and pendimethalin has half-life period of 87 and 40 days with leaching potential index of 129 and 5, respectively in sandy loam soils. Diclosulam inhibits the acetolactate synthase, a keyenzyme responsible for biosynthesis of branched chain amino acids, which are necessary for cell division at meristematic region of target plants. Further, post-emergence application of cycloxydim reduced the density and dry weight of late emerging grassy weeds due to inhibition of acetyl CoA carboxylase, which is responsible for biosynthesis of fatty acids. The grassy weeds showed necrosis of young seedlings owing to cessation of cell division in cluster bean (Sharma *et al.*, 2017). Pre-emergence application of diclosulam 20 g/ha was found to be effective in controlling purplenut sedge due to increased availability of diclosulam in soil solution as a result of better leaching ability, low absorption coefficient coupled with prolonged half-life period compared to both the formulations of pendimethalin, which might have resulted in

increased absorption of diclosulam by deep rooted mother as well as daughter tubers of *Cyperus rotundus*. Price and Wilcut (2002) stated that diclosulam 27 g/ha has been found very effective in controlling yellow nut sedge up to 65-100% when it was applied alone or in combination with dimethenamide at recommended rates. All pendimethalin treated plots registered significantly higher density and dry weight of sedges due to its poor control of purplenut sedge. Pre-emergence application of diclosulam 20 g/ha supplemented with 1 HW at 30 DAS or cycloxidim 100 g/ha at 20 DAS resulted in maximum weed control efficiencies of 94.8 and 93.5%, respectively. Pre-emergence application of diclosulam 20 g/ha *fb* haloxyfop-P-ethyl 135 g/ha considered as next best weed management practice in recording lower density and dry weight of total weeds as well as weed control efficiency.

Growth parameters: All the pre-emergence and post-emergence herbicides tried did not show any phytotoxicity at their recommended rates on groundnut at 10th and 5th day after application, respectively, however pre-emergence application of diclosulam 20 g/ha resulted in phytotoxicity rating of "1" on a 0-10 scale with yellowing of young leaves followed by necrotic patches leading to stunted growth. The crop was recovered from phytotoxicity of diclosulam within 25 days after its application. The groundnut crop might have escaped from the severe phytotoxicity due to bigger size of the kernel whereas weed seeds are smaller in size and would have been susceptible to diclosulam. Pre-emergence application of both the formulation of pendimethalin did not have any phytotoxicity in groundnut. The initial and final plant population of groundnut was not influenced significantly, among the treatments. Pre-emergence application of diclosulam 20 g/ha at 20 DAS *fb1* HW at 40 DAS resulted in higher leaf area index and dry matter production whereas plants were tall with HW twice at 20 and 40 DAS. This might be due to effective control of all the categories of weeds including purple nutsedge during the critical stage which might have increased the plant height, LAI and number of branches/plant which could have lead to higher dry matter production (Deepa *et al.*, 2017). Further, timely and effective control of weeds is expected to have better availability of moisture, nutrients and solar radiation to the crop plants and thereby increased the dry matter production.

Table 1 Effect of different weed management practices on weed density, weed dry weight and weed control efficiency in groundnut at harvest during winter season of 2018-19

Treatments	Dose (g/ha)	Time of application (DAS)	Weed density (No./m^2)				Weed dry weight (g/m^2)				WCE (%)
			Grasses	Sedges	BLWs	Total	Grasses	Sedges	BLWs	Total	
Pendimethalin (CS)	725	1	4.67 (2.37)	32.33 (5.76)	13.33 (3.78)	50.33 (7.16)	4.81 (2.41)	18.41 (4.40)	20.43 (4.62)	43.65 (6.64)	59.83
Diclosulam	20	1	3.33 (2.06)	11.67 (3.55)	3.00 (1.98)	18.00 (4.33)	2.31 (1.81)	4.30 (2.30)	3.73 (2.17)	10.33 (3.36)	90.49
Pendimethalin fb* hand weeding	725	1 fb 40	4.33 (2.30)	25.33 (5.12)	4.33 (2.30)	34.00 (5.91)	3.84 (2.20)	15.30 (4.03)	10.51 (3.39)	29.66 (5.53)	72.71
Diclosulam fb hand weeding	20	1 fb 40	3.00 (1.98)	3.67 (2.15)	1.33 (1.52)	8.00 (2.99)	1.76 (1.65)	2.74 (1.93)	1.15 (1.46)	5.65 (2.57)	94.80
Pendimethalin fb haloxyfop-P-ethyl	725 fb135	1 fb 20	4.00 (2.23)	31.33 (5.68)	20.00 (4.58)	55.33 (7.50)	3.24 (2.05)	17.04 (4.24)	25.44 (5.14)	45.72 (6.83)	57.93
Diclosulam fb haloxyfop-P-ethyl	20 fb135	1 fb 20	2.67 (1.91)	10.33 (3.36)	3.67 (2.15)	16.67 (4.19)	1.32 (1.52)	3.80 (2.18)	3.83 (2.19)	8.95 (3.13)	91.77
Pendimethalin fb cycloxydim	725 fb100	1 fb 20	3.67 (2.15)	27.33 (5.31)	17.67 (4.32)	48.67 (7.04)	2.81 (1.95)	16.25 (4.15)	22.84 (4.88)	41.91 (6.54)	61.43
Diclosulam fb cycloxydim	20 fb100	1 fb 20	2.00 (1.73)	9.33 (3.20)	2.67 (1.91)	14.00 (3.86)	0.90 (1.37)	3.23 (2.05)	2.88 (1.96)	7.01 (2.82)	93.55
Pendimethalin (EC)	1000	1	5.67 (2.57)	62.67 (7.95)	7.67 (2.94)	76.01 (8.75)	6.54 (2.65)	22.43 (4.79)	15.67 (4.02)	44.64 (6.72)	58.92
Hand weedings	-	20 fb 40	9.67 (3.26)	14.67 (3.94)	4.00 (2.22)	28.33 (5.39)	5.30 (2.51)	6.25 (2.69)	10.04 (3.31)	21.59 (4.71)	80.13
Unweeded check			94.33 (9.76)	73.67 (8.63)	56.33 (7.57)	224.33 (15.00)	49.49 (7.10)	31.30 (5.67)	48.53 (7.00)	108.66 (10.45)	-
SEm ±			0.112	0.161	0.098	0.224	0.078	0.108	0.112	0.227	-
CD (P = 0.05)			0.34	0.47	0.28	0.64	0.23	0.32	0.33	0.69	

CS: Capsulated Suspension EC: Emulsifiable Concentrate fb*: followed by DAS: Days after sowing WCE: Weed control efficiency.
The figures in parentheses indicate square root transformed values

Table 2 Effect of different weed management practices on growth, yield attributes yield of groundnut during winter season of 2018-19

Treatments	Dose (g/ha)	Time of application (DAS)	Plant height (cm)	LAI	DMP (kg/ha)	Matured Pods/plant	100-pod weight (g)	100-kernel weight (g)	Pod yield (t/ha)	Haulm yield (t/ha)	Net returns (₹/ha)	B:C ratio
Pendimethalin (CS)	725	1	26.68	1.90	4885	11.70	96.50	41.37	1.55	2.57	34625	2.04
Diclosulam	20	1	25.41	2.07	5445	13.63	103.73	44.51	1.76	2.80	44028	2.35
Pendimethalin fb* hand weeding	725	1 fb 40	27.64	1.95	5071	12.20	98.90	42.82	1.60	2.61	34399	1.97
Diclosulam fb hand weeding	20	1 fb 40	25.85	2.20	5765	15.80	115.87	48.87	2.10	3.05	56485	2.62
Pendimethalin fb haloxyfop-P-ethyl	725 fb135	1 fb 20	27.87	1.99	5122	12.75	100.30	43.94	1.65	2.68	36223	2.02
Diclosulam fb haloxyfop-P-ethyl	20 fb135	1 fb 20	25.93	2.12	5526	14.32	106.20	45.92	1.86	2.85	46058	2.32
Pendimethalin fb cycloxydim	725 fb100	1 fb 20	28.45	2.01	5218	13.30	103.20	44.14	1.70	2.73	39487	2.14
Diclosulam fb cycloxydim	20 fb100	1 fb 20	26.59	2.16	5645	14.80	109.50	46.94	1.93	2.91	49920	2.47
Pendimethalin (EC)	1000	1	27.29	1.95	5062	13.33	98.10	43.20	1.62	2.62	37239	2.12
Hand weedings	-	20 fb 40	29.53	2.03	5357	13.83	113.90	47.35	1.81	2.81	41127	2.10
Unweeded check (Control)			20.73	1.66	4097	9.80	87.56	37.41	1.08	1.95	15631	1.50
SEm ±			1.29	0.09	183	0.34	2.0	1.3	0.8	0.105	1825	0.04
CD(P = 0.05)			3.81	0.28	544	1.14	5.94	3.80	0.24	0.31	5424	0.14

CS: Capsulated Suspension; EC: Emulsifiable Concentrate ; *fb: followed by ; DAS: Days after sowing ; LAI: Leaf area index.
DMP: Dry matter production; B:C Ratio : Benefit-cost ratio

Yield attributes and yield: All the weed management practices significantly influenced the yield attributes and yield of groundnut (Table 2). The highest number of matured pods/plant, 100-pod and kernel weight including pod and haulm yield were recorded with pre-emergence application of diclosulam 20 g/ha supplemented with HW at 40 DAS, which was comparable with pre-emergence application of diclosulam 20 g/ha *fb* cycloxydim 100 g/ha at 20 DAS. Sequential application of diclosulam 20 g/ha supplemented with HW at 40 DAS or cycloxydim 100 g/ha at 20 DAS resulted in broad-spectrum weed control which might have created favourable environment for increased number of matured pods/plant and maintenance of better source sink relationship for good filling of the seeds. The pod yield of groundnut was increased by 48.8 and 44.2% with pre-emergence application of diclosulam 20 g/ha supplemented with HW at 40 DAS and pre-emergence application of diclosulam 20 g/ha *fb* cycloxydim100 g/ha applied at 20 DAS compared to un-weeded check.

The increased pod yield with above weed management practices was mainly due to enhanced number of matured pod /plant and well filled pod and kernel weight as a result of season-long weed control. These results are in agreement with the findings of Grey and Wehtie (2005). The highest net returns and benefit-cost ratio were obtained with pre-emergence application of diclosulam 20 g/ha supplemented with HW at 40 DAS and pre-emergence application of diclosulam 20 g/ha *fb* cycloxydim 100 g/ha applied at 20 DAS due to increased economic yield and reduced cost of weeding. Hand weeding twice at 20 and 40 DAS exhibited lower net returns than above said treatments due to increased cost of weeding, owing to increased labour wages.

Thus, it could be concluded that the highest pod yield and maximum net returns, besides broad-spectrum weed control in groundnut were obtained with pre-emergence application of diclosulam 20 g/ha supplemented with 1HW at 40 DAS, which was comparable with pre-emergence application of diclosulam 20 g/ha *fb* cycloxydim 100 g/ha applied at 20 DAS. All the herbicides tested did not show any phytotoxicity on groundnut growth and development at their recommended rates. It is concluded that whenever the labour availability for supplemental hand weeding is abundant and cheaper, one can go for former weed management practice, otherwise opt for latter weed management practice in controlling mixed weed flora associated with *rabi*-summer groundnut in sandy loam soils.

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Mycorrhizal responsiveness to different phosphorus levels on yield attributes and mycorrhizal colonization of groundnut genotypes

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ABSTRACT

This study examined P bioavailability, groundnut response to applied P and compared P management strategy with AM fungal treatment combinations. A field experiment was carried out at North eastern agro climatic zone of Tamil Nadu during 2018 and the experimental soil was pale brown sandy loam in texture (TypicHaplustalf). The treatments consisted of four levels of phosphorus *viz.,* P_0, P_{25}, P_{50} and P_{75} kg/ha. The availability of P was investigated in the presence (V1) and absence (V0) of AM Fungi. Two genotypes namely VRI-2 (Vridhachalam-2) and G-5 (Gujarat-5) were selected for P growth study in the experiment. The results obtained from the study indicated that, AM inoculated plot was superior in yield potential than uninoculated plots. The treatments showed significant effect on growth and yield of groundnut varieties. The interaction effect of P levels and AM inoculation on growth and yield of groundnut genotypes were significant.

Keywords: AM fungi, Endotrophic, Genotypes, Inoculated, Mycorrhizae

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop because of high oil (46%) and protein (24%) content. It is one of the species of legume family grown and consumed in all parts of the country (Anyasor *et al.*, 2009; Salunke *et al.,* 2018). Phosphorus is the second major essential nutrient element indispensable for normal crop growth, quality, yield and its major effect is exhibited on plant root system development (Ajay *et al.*, 2018). Legumes require high amounts of phosphorus for nodule formation and fixation of atmospheric nitrogen (Brady and Weil, 2002). Phosphorus is a component for energy transfer (ATP), phospholipids and nucleic acids (Sultenfuss and Doyle, 1999). Phosphorus is a constituent of nucleoproteins and phytins essential for cell division and development and in root and nodule formation (Amruth *et al.*, 2018). The critical limit for P is 0.2 % of dry weight. The plant-available P is very low in the soil due to its high reactivity with free iron and aluminium ions especially in acid soils and P forms calcium phosphate in alkaline soils leading to low soil available P (Schachtman *et al.*,1998). Moreover, there is higher demand of P in initial stages of plant growth leading to rapid absorption of P which in turn creates P depletion zones around the rhizosphere. Due to the low mobility of phosphate ions, even high phosphorus soil is unable to replenish the depleted zone rapidly. In India 49.3% of cultivable land area are low in available P (Hasan, 1996). It has been reported that P uptake by plants is mainly through diffusion against high cellular concentration. This process demands high metabolic energy and high affinity transporter in phagosomal transporter (Pht) family (Bucher, 2007). AM fungi is an endotrophic type of mycorrhizae with wide adaptability to extreme environments such as high to low temperatures, high-salt concentrations, and acidic to basic soil conditions. Mycorrhizae can transfer phosphorus via hyphal tube more than four times higher than that of direct uptake (Epstein and Bloom, 2004). The present study investigated the P availability in soil, crop response to applied P and P management strategy by AM fungi in groundnut.

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MATERIALS AND METHODS

The field experiment was carried out at North Eastern agro climatic zone of Tamil Nadu located 79° 65' N longitude 11° 61' E latitude at an altitude of +6 m above MSL. The experimental soil was sandy loam (Typic Haplustalf) with pH 5.8 and EC of 0.27 d/Sm. The soil was low in organic carbon (2.6 g/kg), low in alkaline $KMnO_4$-N (196 kg/ha-), low in Bray-1-P (9.5 kg/ha) and medium in NH_4OAc-K (232 kg/ ha). The treatments consisted of four levels of phosphorus *viz.,* P_0, P_{25}, P_{50} and P_{75} kg/ha. Availability of P in the soil was evaluated both in the presence (V1) and absence (V0) of AM. Eight treatment combination were: T1 (Control), T2 (AM fungi @ 10kg/ha), T3 (P_2O_5 @ 25kg/ha), T4 (P_2O_5 @ 25 kg/ha + AM fungi @ 10 kg/ha), T5 (P_2O_5 @ 50 kg/ha), T6 (P_2O_5 @ 50 kg/ha + AM fungi @ 10 kg/ha), T7 (P_2O_5 @ 75 kg/ha), T8 (P_2O_5 @ 75 kg/ha + AM fungi @ 10 kg/ha).

The sources of nitrogen, phosphorus and potassium were urea, single super phosphate, and muriate of potash respectively. 10 kg of Symbion plus AM fungi mixed with 100 kg of FYM was applied to the field. The experiment was conducted in factorial randomized block design with three replications. Two groundnut genotypes namely VRI-2 (Vridhachalam2) and G-5 (Gujarat-5) were grown as test

crops in two separate experiments. However, results are presented in a single table for comparison of treatment effect on genotypes. Biometric observation of growth and yield characters *viz.,* dry matter production, kernel and oil yield was recorded. Mycorrhizal root colonization was recorded using root staining method (Phillips and Hayman, 1970). Five randomly chosen plants in each plot were tagged to record biometric observations. Periodically root samples were also collected for analysis. The critical differences between the treatments were worked at five percent confidence level by adopting standard statistical procedures.

RESULTS AND DISCUSSION

Dry matter production: Dry matter production of a crop mainly depends on the major nutrient uptake and assimilation. Mycorrhiza un-inoculated plot with 75 kg P/ha recorded maximum dry matter production in 30, 60 and 90 days after planting *viz.,* 1822, 3090 and 6498 kg/ha in VRI-2 and 1809, 2663 and 5359 kg/ha for G-5 genotype respectively. Higher P application maintains soil solution concentration compared to other phosphorus treatments. Lower yield obtained with G-5 variety compared to VRI-2 could be due to varietal character. With respect to the combination treatments of AM and phosphorus at different levels, AM application with P @ 50 kg P_2O_5/ha recorded maximum yield compared to other treatment combinations. The dry matter recorded were 2341 and 2100, 3222 and 3112, 6701 and 5992 kg/ha in 30, 60 and 90 days after sowing of VRI-2 and G-5 varieties respectively (Table 1). The control without phosphorus and AM inoculation recorded the lowest dry matter values at all stages of crop growth. Higher uptake and assimilation of N and P due to mycorrhizal fungi treatment could be due to the increased ability of the crop to photosynthesise leading to increased accumulation of dry matter. Phosphorus acts as an energy base which enhances the translocation of sugars and carbohydrates to all parts of the plants. The results obtained are in accordance with findings of Smith and Read (2008) and Kumar *et al*. (2014). Further, the increased growth characters of groundnut with increasing levels of P indicated the positive impact of phosphorus availability which improved the overall plant growth and enhanced root proliferation which could be attributed to adequate phosphorus nutrition. This ultimately led to better absorption and utilization of nutrients from soil solution, reflecting in improved plant performance. Thus, the increased growth parameters due to improved nutritional environment observed in the present study confirmed the results reported by Mandhata *et al*. (1994).

Kernel yield and oil yield: Kernel and oil yield of groundnut, as in any other crop, is mainly based on the proportion of nutrient uptake, accumulation and transportation of nutrients. Among various treatment combinations T6 - P @ 50 kg/ha + AM inoculation recorded highest kernel and oil yield of 2422 and 1136 in VRI-2 and 2005 and 949 in G-5 respectively (Table 3). Oil content did not change due to the treatments in both the varieties but the oil yield did differ significantly due to the change in the pod yield. The treatment T8 was inferior compared to T6, due to increase in P levels which perhaps restricted the mycorrhizal colonization leading to reduced nutrient uptake and assimilation. The increase in yield and yield characters of groundnut with application of phosphorus @ 50 kg/ha level was reported by Sharma and Yadav (1997) and Gobarah *et al*. (2006). Ha (2003) reported that application of 60 kg P/ha gave significantly higher yield than control in alluvial soil while 90 kg P/ha gave significantly higher yield in sandy soil. This observation suggests that the increased nutrient uptake perhaps leads to increased photosynthesis by higher water and nutrient use efficiency. This in turn could enhance the plant's ability to produce more assimilates which get reflected in the kernel and oil yield. Similar results have also been reported by many workers (Tomar *et al*., 1997; Gobarah *et al*., 2006).

Mycorrhizal root colonization: The higher AM fungal colonization was recorded in P0V1 treatment across the three developmental stages *viz.,* 30 DAS (31.24 and 28.6 per cent), 60 DAS(51.01and 46.3per cent) and 90 DAS (56.81 and 50.07 per cent) in both VRI-2 and G-5 groundnut genotypes (Figure 1). The lowest colonization was recorded in P75V0. P application significantly is known to reduce the AM fungal population. The descending trend of mycorrhizal root colonization as the phosphorus level increased may be due to the decrease in production and secretion of mycorrhizal stimulants (strigolactone) by the plants leading to lower colonization of roots by the fungus (Tamasloukht *et al.,* 2003; Besserer *et al.,* 2007; Jamil *et al.,* 2011).

Soil available phosphorus: The values of soil available phosphorus increased with the increased levels of phosphorus application (Table 2) irrespective of the fungal treatment suggesting increased P in soil solution (Paulter and Sims, 2000). Higher soil available phosphorus was recorded in P75V1 on 30 DAS in both VRI-2 and G-5 genotypes. With increase in days, soil available P decreased significantly which could be due to the crop uptake and fixation by the soil. Soil available P were higher in AM inoculated plots compared to AM non-inoculated plots in both the genotypes at 30, 60 and 90 DAS and this may be due to better mycorrhizal root association.

The results of the study clearly indicated that groundnut responded well to different levels of P fertilizer application. Increase in the level of P from 0 to 75 kg/ha significantly increased the growth components of groundnut. Addition of P upto 50 kg/ha along with AM inoculation @10 kg/ha registered a significantly higher growth and yield

components such as dry matter production, kernel and oil yield of groundnut in both varieties VRI-2 and G-5 compared to all other treatments. Mycorrhizal root colonization increased with decrease in level of phosphorus application.

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Table 1 Effect of phosphorus fertilization and mycorrhizal inoculation on dry matter production (kg/ha) of groundnut genotypes

AM levels/ P levels (kg/ha)	VRI-2									G-5								
	30 DAS			60 DAS			90 DAS			30 DAS			60 DAS			90 DAS		
	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean
P_0	1218	1399	1308	2224	2411	2317	4950	5281	5115	1328	1461	1394	1901	2067	1984	3860	4192	4026
P_{25}	1498	1630	1564	2551	2754	2652	5566	5892	5729	1518	1639	1578	2171	2355	2263	4459	4776	4617
P_{50}	1701	2341	2021	2892	3512	3202	6158	7218	6688	1701	2100	1900	2483	3112	2797	5042	5992	5517
P_{75}	1822	1910	1926	3090	3222	3156	6498	6701	6599	1809	1882	1845	2663	2766	2714	5359	5598	5478
Mean	1559	1820	1689	2689	2974	2867	5793	6273	6033	1589	1770	1679	2304	2575	2439	4680	5139	4909
Factor	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P
CD	39.0	55.2	78.0	92.8	131.3	185.7	173.7	245.6	347.7	41.9	59.3	83.9	49.7	70.2	99.4	113.3	160.3	226.7
SED	18.1	25.7	36.3	43.3	61.2	86.6	81.0	114.5	162.0	19.5	27.6	39.1	23.1	32.7	46.3	52.8	74.7	105.6

30, 60 and 90 DAS (Days after sowing) P_0- Phosphorus @ 0 kg/ha; P_{25}- Phosphorus @ 25 kg/ha
P_{50}- Phosphorus @ 50 kg/ha; P_{75}- Phosphorus @ 75 kg/ha

Table 2 Effect of phosphorus fertilization and mycorrhizal inoculation on Soil available phosphorus (kg/ha) of groundnut genotypes

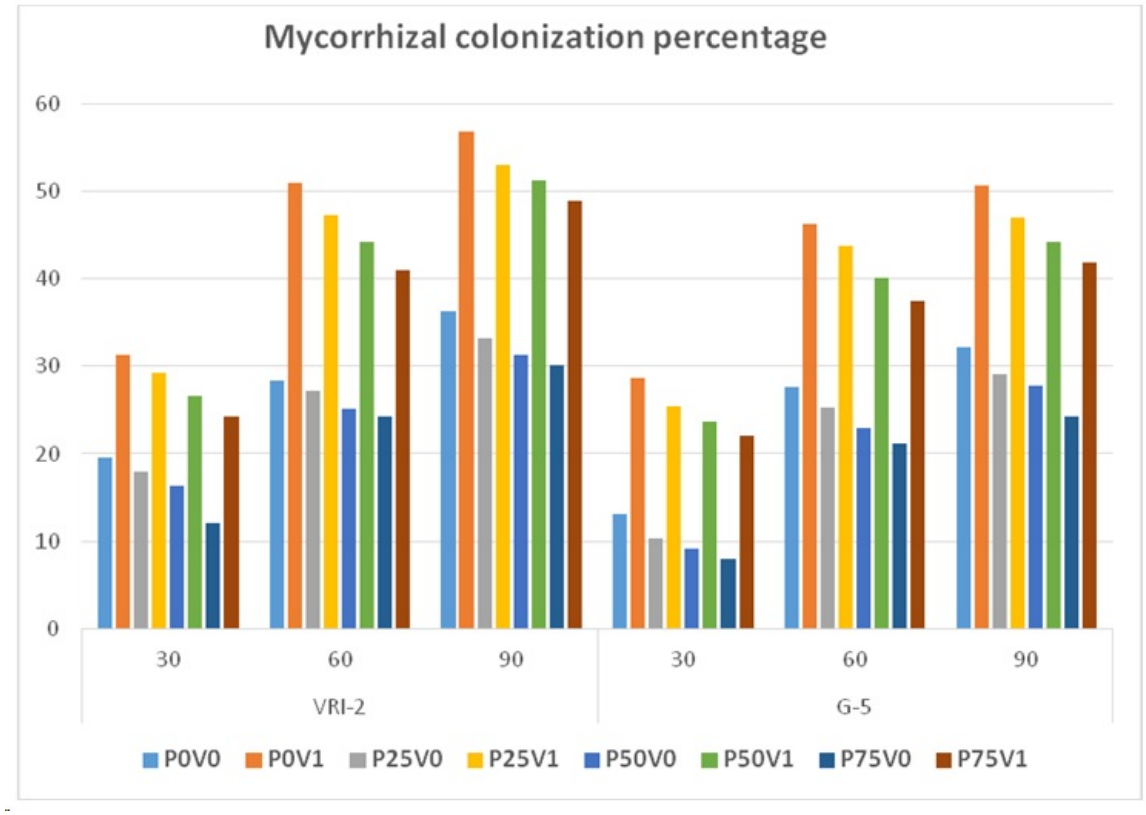
AM Levels /P Levels (kg/ha)	VRI-2									G-5								
	30 DAS			60 DAS			90 DAS			30 DAS			60 DAS			90 DAS		
	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean	V0	V1	Mean
P_0	15.6	18.3	17.0	11.6	14.1	12.9	8.2	11.3	9.8	17.9	19.6	18.8	14.5	17.2	15.9	10.3	14.9	12.6
P_{25}	22.3	24.5	23.4	20.5	21.3	20.9	13.6	15.2	14.4	24.8	27.3	26.1	22.6	21.7	22.2	16.2	18.4	17.3
P_{50}	26.9	29.5	28.2	23.7	25.4	24.6	17.4	19.1	18.3	29.2	31.2	30.2	24.5	26.6	25.6	19.3	22.1	20.7
P_{75}	32.1	34.8	33.5	29.8	30.9	30.4	22.6	24.9	23.8	34.1	36.4	35.3	28.4	31.7	30.1	23.8	25.1	24.5
Mean	24.2	26.8	25.5	21.4	22.9	22.2	15.5	17.6	16.5	26.5	28.6	27.6	22.5	24.3	23.4	17.4	20.1	18.8
Factor	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P	V	P	V X P
CD (0.05)	0.56	0.80	1.13	0.64	0.91	1.28	0.35	0.50	0.70	0.60	0.85	1.21	0.59	0.83	1.18	0.49	0.69	0.98
SED	0.26	0.37	0.53	0.30	0.42	0.61	0.16	0.23	0.33	0.28	0.49	0.56	0.27	0.39	0.55	0.23	0.32	0.46

30, 60 and 90 DAS (Days after sowing) P_0- Phosphorus @ 0 kg/ha; P_{25}- Phosphorus @ 25 kg/ha
P_{50}- Phosphorus @ 50 kg/ha; P_{75}- Phosphorus @ 75 kg/ha

Table 3 Effect of phosphorus fertilization and mycorrhizal inoculation on Kernel and oil yield of groundnut genotypes

Varieties / Treatments	VRI-2				G-5			
	Kernal yield (kg/ha)		Oil yield (kg/ha)		Kernal yield (kg/ha)		Oil yield (kg/ha)	
P0V0	1350		636.7		1165		514.5	
P0V1	1473		702.3		1293		576.8	
P25V0	1554		746.2		1360		614.4	
P25V1	1695		819.1		1466		668.0	
P50V0	1764		864.1		1553		711.4	
P50V1	2242		1136.4		2005		949.3	
P75V0	1882		927.4		1677		774.6	
P75V1	1976		990.7		1733		809.6	
	P	V	PV	P	V	PV	P	V	PV	P	V	PV
CD (0.05)	55.3	39.1	78.2	9.3	6.6	13.2	36.8	26.0	52.1	20.7	14.6	29.3
SED	25.7	18.2	36.4	4.3	3.0	6.1	17.2	12.1	24.3	9.6	6.8	13.6

(P_0, P_{25}, P_{50} and P_{75} - 0, 25, 50 and 75 kg of P/ha ; V_0 - AM Non inoculated and V_1 - AM inoculated)



(P_0, P_{25}, P_{50} and P_{75} - 0, 25, 50 and 75 kg of P/ha ; V0 - AM Non inoculated and V1 - AM inoculated)
Fig. 1. Effect of phosphorus fertilization and mycorrhizal inoculation on AM fungal Root colonization (%) of groundnut genotypes

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Fat quality of ready to eat foods without nutritional label from unorganized sector

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ABSTRACT

The aim of this study was to investigate total fat and fatty acid composition in processed foods from unorganized sector (without nutritional labels), with emphasis on trans fatty acid (TFA) content. A total of 41 products, divided into 5 categories, *viz.,* salted snack foods (n=9), sweets (n=7), street foods (n=10), meal items (n=4) baked foods (n=11) were sampled for estimation of fatty acid composition. Results indicate mean total fat content in all foods ranged between 16.88 to 30.64%, with high saturated (36.14 to 58.20%) and trans fat content (1.18 to 3.40%). Palmitic acid (C16:0) was the highest fatty acid varying between 17.01% - 46.80% in all the processed foods, followed by Oleic acid (C18:2, n6cis). Elaidic acid (C18:1, n9trans), Linolelidic acid (C18:2, n6trans) and Vaccenic acid (C18:1, n11trans) were the trans fatty acids found in 93% of all the foods estimated. The quality of fats in most of the foods analyzed was found to be unhealthy due to high TFA content, which is a possible contributing risk factor for diet-related non-communicable diseases, and the consumers are unaware of the fat quality due to absence of nutritional labels.

Keywords: Fat, Fatty Acids, Processed RTE food, Nutritional label, Unorganised sector

India's food retail business is dominated by a large number of small retailers consisting of the local kirana shops, food stores, owner manned general stores, hand-cart hawkers, pavement vendors, street food carts etc. which together make up the "unorganized retail sector" (Vidushi and Grover, 2012). Food processing industry in India is highly fragmented and dominated by unorganized sector, predominantly small, family owned businesses (Singh, 2007). Most of the players in this unorganized industry are small. A study by Fisseha *et al.* (2012) reported that about 42% of the processed food comes from the unorganized sector, whereas 25% comes from the organized sector. Another study by Vidushi and Grover (2012) reported that around 96% of Indian retail sector (food and non food products) is from small to medium players with majority of sales taking place through unorganized stores popularly known as kirana or 'mom-and-pop' stores. Though the unorganized segment varies across categories, approximately 75% of the market sales in India is still in this segment. Though organized sector is growing at a faster rate, unorganized sector is still preferred for food purchases by the customers due to the convenience and easy approachability they offer (Fisseha *et al.*, 2012).

A study by Fisseha *et al.* (2012) reported that various kinds of food products are regularly procured from the unorganized sector operated by vendors selling grains, snack foods, street food, ready-to-eat foods, sweets, baked products, etc. Majority of these foods do not have nutrition labels nor they are registered under any food act. Hence, any consumer who buys these foods is unaware of nutritional quality, food label or the standard of the food, which is a major constraint in processed, ready to eat foods purchased from unorganized sector (Vidushi and Grover, 2012).

Recent report from FSSAI (2017) states that, on an average Indians consume approximately 47.9 to 57.1 g/d of total fats which is higher than the recommended intake of total and saturated fats. The increasing trends were reported for men and women from both urban and rural settings and sources of high fat intake was due to increased consumption of out-of-home food, processed and fried foods etc. Average daily consumption of processed foods (g or ml /day) among urban population was higher in high income groups with a mean intake of 18.93 g/d fried snacks and 12.2% sweets, whereas the mean intake of chat items (savory snacks, largely from roadside sources) was 10.18% among low income group. Consumption of processed foods (g or ml/day) among urban population in India by socioeconomic group was high among low income group with consumption of fried snacks (75 g/d) and sweets (44.3 g/day), whereas, chat items (42.3 g/day) were highly consumed among industrial labour workers. Bakery items (60 g/day) was highly consumed in slums.

India is facing an "epidemic" of diet-related non-communicable diseases (DR-NCDs). A decreasing intake of coarse cereals, pulses, fruits and vegetables; an increasing intake of fat, salt and sugar from processed foods, coupled with declining levels of physical activity due to rapid urbanization have all resulted in escalating levels of obesity, atherogenic dyslipidemia, subclinical inflammation, metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease in Indians (Misra *et al.,* 2011; Rizwana Shaik *et al.,* 2018). As diet is an important risk factor for DR-NCDs, understanding the pattern of dietary intake, especially fat intake may provide clues to the causes of these increases in chronic conditions. Fatty acids generally enter the human

diet as by-products of hydrogenation of polyunsaturated fats, and several studies have reported an association between consumption of saturated and trans fatty acids and risk of obesity and cardiovascular disease (CVD) (Shauna *et al.,* 2014, 2009; Mozaffarian *et al.,* 2010; WHO, 2013).

The epidemic of obesity and CVD in most countries has led to public health emphasis on low fat, low energy diets and also for development of low fat products and fat substitutes. However, recent research suggests that there is a definite need to consider the quality as well as the quantity of fat being consumed, as the issues of fat qualityon potential adverse effects of saturated and trans unsaturated fatty acids on circulating cholesterol concentrations remain as most important aspect of public health nutrition (Shauna *et al.*, 2015, Mozaffarian *et al.,* 2010). In India, consumption of poor quality fat is mostly through the foods consumed from unorganized sector, which do not contain any nutritional label. In order to quantify the level of total fat and fatty acid consumption along with trans fats in widely consumed processed food items from unorganized sector, we measured the total fat, saturated fat, and the trans fatty acid content in 41 food items.

MATERIALS AND METHODS

A survey was conducted in the cities of Hyderabad and Secunderabad, India for selecting the most commonly consumed processed foods from the unorganized sector which did not consist of any nutrition label, while purchasing. A standardized questionnaire was prepared with a list consisting of 75 processed food products (distributed in 5 categories) commonly consumed. There were five different categories of foods namely, salted snack foods, sweets, street foods, meal items and baked foods, consisting of 15 foods commonly consumed in each category. The standardized questionnaire was provided to 100 subjects, who were asked to tick the commonly procured processed foods from the market. Purposive sampling technique was used to select the subjects, where a subject was willing to participate and answer the questionnaire after explaining about the objective of the survey. The questionnaire was given to them and they had to answer whether they purchased the foods or not (Yes or no) with a frequency of at least once in a month. A food which was purchased by at least 25% of the subjects was selected for analysis, with a hypothesis that atleast 25% of the population was purchasing that particular food product. A total of 41 food products were selected (Table 1) for analysis of total fat and fatty acid composition including trans fatty acids. Each food product was purchased from four different locations (East, West, South and Northern parts of the twin cities) and the all the four samples were homogenized. The homogenized sample was used for analysis of fat and fatty acids.

Table 1 Foods selected for fat and fatty acid composition

Food Category	Food items selected	Description of the food category
Salted snack foods (n=9)	Gathi, Mathri, Namak para, Boondi, Chegodi, Muruku, Chuduwa, Sakinalu and Sev	Prepared with either rice flour or gram flour or refined wheat flour which were deep fried in oil.
Sweets (n=7)	Mysorepak, Milk Mysorepak, Gulabjamun, Arise, Soanpapdi, Pheni, and Jalebi	Prepared with refined wheat flour / rice flour along with major ingredients like sugar and fat.
Street foods (n=10)	Samosa, Vegetable Curry Puff, Pakodi, MirchiBajji, PaniPuri, Kachori, Punugulu, Potato Chips, Tapioca Chips and Banana Chips	Deep fried foods prepared with refined wheat flour or gram flour or rice flour or black gram dhal, and chips were deep fried products prepared from potato, tapioca and raw banana.
Meal items (n=4)	Chicken Biryani, Mutton Biryani, Beef Biryani and Haleem	Fat and energy dense meal items prepared with cereal, meat and fat and are consumed very frequently.
Baked Items (n=11)	Fruit biscuits, Osmania biscuits, Chai biscuits, Chand biscuits, Salt biscuits, Khari biscuits, Fine biscuits, Bun, Biscuits, Masala kulcha and Cake	Prepared from refined wheat flour and fat, which are generally consumed with tea or as snack item.

Total fat and fatty acid analysis: Total fat content was analysed in moisture free samples byGerhardt soxtherm fat analyser (AOAC 2003.06, 20th Edition). Fatty acids were analysed by AOAC (2001. 996.06) and Geetha *et al.* (2016) methods. The isolated fat was trans-esterified using 0.5 M methonolic KOH to form fatty acid methyl esters (FAME). Fatty acids were estimated by Gas Chromatograph (7890B of Agilent Technologies) equipped with flame ionization detector and Agilent - DB-FFAP column (nitroterephthalic-acid-modified polyethylene glycol (PEG) of high polarity for the analysis of volatile fatty acids). The temperature of the column was maintained at initial temperature of 100°C for 5 min, raised to 240°C at the rate of 4°C/min. Nitrogen was used as carrier gas at a column flow rate of 1.0 ml/min. Detector temperature was maintained at 280°C. Standards used were 47885-U Supelco® 37 Component FAME Mix,

10 mg/mL in methylene chloride. Individual trans-fatty acid standards, Supelco trans-9-Eliadic methyl ester, 10 mg/ml in heptane, trans-9, 12-Octadecadienoic (linoleliadic) methyl ester and trans-11-Vaccenic methyl ester, were used. Sample fatty acid composition was compared with standard fatty acid composition and percentages were calculated by normalization of peak areas. The total fat content and fatty acid composition data was subjected to calculation of mean, ranges, chi-square test and p-values. F-ratio was calculated to check the level of significance at p <0 .05.

RESULTS AND DISCUSSION

Results of fatty acid composition, given in Table 2, indicate that the total fat content varied between 19.27 - 48.55 g/100gms in salted snack foods with a mean value of 30.64g/100gms. Total unsaturated fatty acid content (UFA) was highest in the salted snack foods with mean UFA content of 59.89%, followed by saturated fatty acid (SFA) (36.53%) and trans fatty acid (TFA) (3.40%). The total mean fat content of sweets was 24.15g/100gms, with highest SFA (58.20%) followed by UFA (38.98%) and 2.24% TFA. The street foods had a total mean fat content of 26.33g/100gms with highest UFA (62.06%) and TFA (4.20%). The meal products had a total fat content of 20.47g/100gms with highest UFA (62.68%) and 1.18% TFA. Baked foods had a total fat content of 16.88g/100gms with 51.12% SFA, 45.53% UFA and 3.32% TFA. All the food categories had TFA content between 1.18 % to 4.20%, which could be due to addition of fats, shortenings, margarines, hydrogenated fats etc to contribute individual functional properties to the foods (Reshma *et al.,* 2012; Rizwana *et al.,* 2018).

Among the SFA in salted snack foods, palmitic acid (C16:0) was the highest varying between 17.01% - 46.80% (Table.3). Stearic acid (C18:0) was found to be in the range of 0.57% - 6.98%. Sweets also had highest amount of palmitic acid (C16:0) (35.16 - 50.00%), stearic acid (C18:0) (4.87 - 14.59%) and Myristic acid (C14:0) (0.66 - 10.73%). There was similar trend in the street foods (6.65 - 47.20%) and meal products (14.49 - 35.80%) as well with highest amount of palmitic acid. Baked foods also had highest amount of palmitic acids (23.30 - 50.10%) and stearic acid (1.01 - 7.53%). Other SFA like Myristic acid (C14:0) Lauric acid (C12:0) and Arachidic acid (C20:0) were found in traces along with Behenic acid (C22:0) and Capric acid (C10:0) in all the categories of processed foods. The permitted RDA from SFA for Indians is 10% of total fat as per NIN recommendations (2010), whereas the mean SFA content in all the product categories ranged between 33.57% to 58.20% of total fat which was higher than that of the RDA.

Table 2 Mean and range of total fat, SFA, UFA and TFA of the five categories of samples studied

	Total Fat (%)	Total SFA (% of Total Fat)	Total UFA (% of Total Fat)	Total TFA (% of Total Fat)
Salted Snack Foods	30.64 (19.27 - 48.55)	36.53 (20.50 - 48.49)	59.89 (37.20 - 74.82)	3.40 (0.00-15.13)
Sweets	24.15 (4.40 - 51.20)	58.20 (44.79 - 68.08)	38.98 (28.99 - 55.02)	2.24 (0.00 - 11.77)
Street Foods	26.33 (21.00 - 33.65)	33.57 (9.81 - 54.68)	62.06 (32.70 - 89.35)	4.20 (0.68 - 15.90)
Meal	20.47 (13.19 - 28.82)	36.14 (21.95 - 45.83)	62.68 (52.87 - 77.08)	1.18 (0.98 - 1.40)
Baked	16.88 (1.20 - 27.00)	51.12 (33.49 - 57.05)	45.53 (33.60 - 65.09)	3.32 (0.20 - 15.46)

The f-ratio value is 37.88. The p-value is < .00001. The result is significant at p < .05. SFA - Saturated Fatty Acids; UFA - Unsaturated Fatty Acids; TFA - Trans Fatty Acids.

Vegetable fat from processed foods is increasing in many countries, including India, much of which is from palm oil (Baker and Friel, 2014). Palm oil has become ubiquitous in the entire Indian food supply chain due to the government subsidy on its import and supply of palm oil through the public distribution system (Government of India, 2014). As we can see from the results, the overall fat content in all the categories of food samples studied had very high amount of fat content (16.88% to 30.64%). Among the fatty acids, palmitic acid (C16:0) content was highest, indicating rampant usage of palm oil and vanaspati for preparation and processing of the foods by the food vendors. Palm oil, which is high in saturated fat is used as a replacement oil due its semisolid texture, which is comparable to partially hydrogenated vegetable oils and butter, and its low cost (Downs *et al.,* 2012), and its consumption has been associated with increased risk of NCDs (Chen *et al.,* 2011).

Replacement of dietary SFA with polyunsaturated fats has been shown to have beneficial effect on Coronary heart disease risk and LDL-C concentrations as per recommendations of American Heart Association (AHA, 2015).WHO has recommended that TFA intake as a % of Energy should not exceed 1%. The total fat intake as a % of Energy should not be less than 15% and should not exceed 30%. The intake of Saturated Fat (SFA) as a % of Energy should not exceed 10% (7% for cardiac patients) (WHO, 2015). TFAs are similar to SFAs in increasing LDL cholesterol but in addition they lower the protective effects of HDL cholesterol and increase the lipoprotein (a) which further increases the CVD risk. Studies on experimental animals (Ghafoorunissa, 2005) and limited data in humans suggest that high intakes of either SFAs and / TFAs may

contribute to insulin resistance whereas PUFAs may prevent insulin resistance (Elmadfa and Kornsteinera, 2009; Melanson *et al.,* 2009; Sanders, 2009; Uauy *et al.,* 2009).

Using the criteria to define the strength of evidence between exposure and disease (convincing, probable, possible and insufficient), WHO/FAO Expert Group on Diet, Nutrition and the Prevention of Chronic Diseases (TRS 916) endorsed that qualitative composition of fats in the diet has a significant role to play in modifying risk factors of CVD and set the following ranges for population nutrient goals (% E): total fat, 15-30 (at least 20 % E is consistent with good health), SFAs, <10%; PUFAs, 6-10; n-6, 5-8; n-3, 1-2; TFAs,<1) (WHO, 2003). However, the results of our study indicate that, intake of ready to eat processed foods from unorganized sector might lead to consumption of higher levels of SFAs and TFAs beyond the prescribed recommendations, due to presence of high SFA and TFA beyond the prescribed limits.

Table 3 Saturated Fatty Acid Composition of foods without nutrition labels from unorganized sector

Name of food	Total fat (%)	Butyric acid (C4:0)	Caproic Acid (C6:0)	Caprylic Acid (C8: 0)	Capric Acid (C10: 0)	Lauric Acid (C12:0)	Myristic Acid (C14:0)	Pentadecanoic Acid (C15:0)	Palmitic Acid (C16:0)	Heptadecanoic acid (C17:0)	Stearic Acid (C18:0)	Arachidic Acid (C20:0)	Behenic Acid (C22:0)
Salted Snack foods													
Gathi	44.62	ND	ND	ND	ND	0.18	0.99	ND	40.55	ND	3.64	0.22	ND
Mathri	19.27	ND	ND	ND	ND	0.18	0.99	ND	35.60	0.11	4.12	0.34	ND
Namak para	22.00	ND	ND	ND	ND	0.59	1.23	ND	44.30	ND	1.55	ND	ND
Boondi	36.00	ND	ND	0.30	ND	ND	ND	ND	17.80	ND	1.70	ND	0.70
Chegodi	23.00	ND	ND	ND	ND	ND	1.12	ND	46.80	ND	0.57	ND	ND
Mukuru	24.20	ND	ND	ND	ND	ND	ND	ND	19.40	ND	5.90	ND	ND
Chudwa	48.55	ND	ND	ND	ND	ND	0.63	ND	17.01	ND	6.98	ND	ND
Sakinalu	21.33	ND	ND	ND	ND	ND	0.14	ND	25.77	ND	5.19	ND	ND
Sev	36.75	ND	ND	ND	ND	0.28	0.97	ND	38.43	ND	4.51	ND	ND
Sweets													
Mysorepak	51.20	ND	ND	ND	1.53	2.30	10.47	1.52	37.66	ND	14.59	ND	ND
Milk Mysorepak	29.30	ND	ND	ND	1.13	1.78	10.73	1.54	35.81	0.83	13.91	ND	ND
GulabJamun	4.40	0.37	1.04	0.86	1.67	4.56	9.13	ND	37.88	ND	11.10	ND	ND
Arise	15.01	ND	ND	ND	1.17	1.95	7.10	ND	35.16	ND	10.03	ND	ND
Soanpapdi	23.87	ND	ND	ND	ND	0.18	0.66	ND	39.08	ND	4.87	ND	ND
Pheni	39.29	ND	ND	ND	ND	0.96	0.88	ND	46.03	ND	5.25	ND	ND
Jalebi	6.00	ND	ND	0.26	ND	0.34	1.38	ND	50.00	1.72	ND	ND	ND
Street foods													
Samosa	21.00	ND	ND	0.26	0.17	0.64	1.80	ND	32.90	ND	1.98	0.39	ND
Punugulu	30.13	ND	ND	ND	ND	ND	0.02	ND	6.54	ND	3.25	ND	ND
Veg. Curry Puff	20.00	ND	ND	ND	0.27	4.12	2.39	ND	46.40	ND	1.50	ND	ND
Pakodi	33.65	ND	ND	ND	ND	0.42	0.07	ND	17.53	ND	3.64	ND	ND
MirchiBajji	28.36	ND	ND	ND	ND	ND	0.16	ND	7.99	ND	3.00	ND	ND
PaniPuri	23.75	ND	ND	ND	ND	0.20	1.00	ND	40.86	0.10	4.39	0.30	ND
Kachori	26.31	ND	ND	ND	ND	ND	0.09	ND	7.03	ND	3.08	0.17	ND
Potato chips	26.62	ND	ND	ND	ND	ND	1.15	ND	41.75	ND	4.22	ND	ND
Tapioca chips	24.00	ND	ND	ND	ND	ND	1.21	ND	47.20	0.88	ND	ND	ND
Banana chips	29.51	ND	ND	ND	ND	0.24	1.05	ND	41.21	ND	3.90	0.27	ND
Meal Items													
Chicken Biryani	19.83	ND	ND	ND	ND	0.20	0.89	ND	35.68	ND	4.59	0.33	ND
Mutton Biryani	20.03	ND	ND	ND	ND	ND	1.20	0.15	14.49	0.25	5.59	0.27	ND
Beef Biryani	13.19	ND	ND	ND	ND	0.21	1.32	0.19	35.80	0.31	7.63	0.37	ND
Haleem	28.82	ND	ND	ND	ND	0.62	2.05	0.23	26.17	ND	6.02	ND	ND
Baked foods													
Fruit Biscuits	18.91	ND	ND	ND	ND	ND	1.09	ND	41.69	ND	7.53	ND	ND
Osmania Biscuits	22.16	ND	ND	ND	ND	3.23	2.44	ND	44.23	ND	6.25	ND	ND
Chai Biscuits	19.67	ND	ND	ND	ND	ND	1.21	ND	47.25	ND	4.52	ND	ND
Chand Biscuits	17.13	ND	ND	ND	ND	3.28	2.3	ND	44.81	ND	6.52	ND	ND
Salt Biscuit	4.16	ND	ND	ND	ND	3.25	2.44	ND	44.67	ND	6.24	ND	ND
Khari Biscuit	26.15	ND	ND	ND	ND	ND	1.29	ND	49.23	ND	6.48	ND	ND
Fine Biscuit	20.22	ND	ND	ND	ND	0.17	1.08	ND	49.90	ND	5.90	ND	ND
Bun	1.20	ND	ND	ND	ND	ND	0.44	ND	23.30	9.75	1.01	ND	ND
Biscuits	27.0	ND	ND	ND	ND	0.97	1.46	ND	50.10	1.04	6.70	ND	ND
Masala Kulcha	11.05	ND	ND	ND	ND	ND	0.91	ND	38.72	ND	4.05	ND	ND
Cake	18.00	ND	ND	ND	ND	0.71	1.19	ND	41.64	ND	1.01	ND	ND

*ND - Not Detected

The results of Table 4 indicate the content of unsaturated fatty acids (UFA) and trans fatty acids in the foods analyzed. Among the UFA, Oleic acid (C18:2n6cis) was the highest fatty acid present, compared to other fatty acids in all the foods analyzed. C18:2n6cis varied between 31.60 - 42.48% in salted snack foods; 24.82 - 43.83% in sweets; 20.40 - 42.82% in street foods; 28.75 - 43.26% in meal items; 33.60 - 43.02% in baked foods. Linolenic acid (C18:3) was also present in fair quantities among the foods analyzed with a range of 0 - 42.80% in salted snack foods; 0 - 11.80% in sweets; 0 - 43.97% in street foods; 11.05 - 45.21% in meal items and 0 - 23.84% in baked foods.

Table 4 Unsaturated and Trans Fatty Acid Composition of foods without nutrition labels from unorganized sector

Name of food	Total fat (%)	Unsaturated Fatty Acids						Trans Fats		
		Myristoleic Acid (C14:1)	Palmitoleic acid (C16:1)	Oleic Acid (C18:1n9cis)	Linoleic Acid (C18:2n6cis)	α-Linolenic acid (18:3)	Eicosenoic Acid (C20:1n9)	Elaidic Acid (C18:1,n9 trans)	Linolelidic acid (18:2,n6Trans)	Vaccenic Acid (C18:1 n11T)
Salted Snack Foods										
Gathi	44.62	ND	ND	38.23	12.38	0.27	0.14	3.38	ND	ND
Mathri	19.27	ND	0.34	34.42	23.51	0.19	ND	ND	0.21	ND
Namak para	22.00	ND	ND	37.20	ND	ND	ND	8.10	7.03	ND
Boondi	36.00	ND	ND	31.60	42.80	ND	ND	4.42	0.41	ND
Chegodi	23.00	ND	ND	40.00	5.56	ND	ND	4.54	0.10	ND
Mukuru	24.20	ND	ND	31.70	42.07	ND	ND	0.95	ND	ND
Chudwa	48.55	ND	ND	38.01	36.82	ND	ND	ND	0.56	ND
Sakinalu	21.33	ND	ND	40.69	27.32	ND	ND	0.89	ND	ND
Sev	36.75	ND	ND	42.48	13.32	ND	ND	ND	ND	ND
Sweets										
Mysorepak	51.20	0.75	2.28	24.82	3.34	ND	ND	ND	ND	ND
Milk Mysorepak	29.30	ND	2.17	28.06	4.05	ND	ND	ND	ND	ND
GulabJamun	4.40	0.65	ND	25.50	2.84	ND	ND	ND	ND	1.69
Arise	15.01	ND	ND	32.24	11.80	ND	ND	ND	ND	ND
Soanpapdi	23.87	ND	ND	43.83	11.19	ND	ND	ND	0.19	ND
Pheni	39.29	ND	ND	37.50	7.35	ND	ND	1.27	0.76	ND
Jalebi	6.00	ND	ND	34.50	ND	ND	ND	7.84	3.93	ND
Street Foods										
Samosa	21.00	ND	ND	20.40	33.82	ND	ND	6.18	0.33	ND
Punugulu	30.13	ND	ND	32.04	57.31	ND	ND	0.85	ND	ND
Veg. Curry Puff	20.00	ND	ND	32.70	ND	ND	ND	8.0	4.11	ND
Pakodi	33.65	ND	ND	33.70	43.97	ND	ND	ND	0.68	ND
MirchiBajji	28.36	ND	0.14	29.16	58.11	0.35	ND	1.10	ND	ND
PaniPuri	23.75	ND	0.15	42.01	9.60	0.13	0.23	0.84	0.18	ND
Kachori	26.31	ND	0.11	29.31	58.53	ND	ND	1.11	0.56	ND
Potato chips	26.62	ND	ND	42.82	9.78	ND	ND	ND	0.27	ND
Tapioca chips	24.00	ND	ND	34.79	ND	ND	ND	10.2	5.70	ND
Banana chips	29.51	ND	ND	40.61	10.61	0.22	ND	1.90	ND	ND
Meal Items										
Chicken Biryani	19.83	ND	0.91	43.26	12.86	0.23	ND	0.88	0.17	ND
Mutton Biryani	20.03	ND	0.46	31.13	45.21	0.28	ND	0.98	ND	ND
Beef Biryani	13.19	ND	0.43	41.14	11.05	0.25	ND	1.12	0.17	ND
Haleem	28.82	0.11	ND	28.75	34.65	ND	ND	1.40	ND	ND
Baked Foods										
Fruit Biscuits	18.91	ND	ND	41.44	7.09	ND	ND	0.90	0.26	ND
Osmania Biscuits	22.16	ND	ND	35.41	7.29	ND	ND	0.99	0.16	ND
Chai Biscuits	19.67	ND	ND	36.79	9.26	ND	ND	0.72	0.25	ND
Chand Biscuits	17.13	ND	ND	35.34	6.92	ND	ND	0.83	ND	ND
Salt Biscuit	4.16	ND	ND	35.43	7.20	ND	ND	0.62	0.15	ND
Khari Biscuit	26.15	ND	ND	36.91	4.51	ND	ND	1.23	0.35	ND
Fine Biscuit	20.22	ND	ND	35.32	7.07	ND	ND	0.27	0.29	ND
Bun	1.20	1.81	ND	39.44	23.84	ND	ND	1.01	ND	ND
Biscuits	27.00	ND	ND	33.60	ND	ND	ND	6.17	ND	ND
Masala Kulcha	11.05	ND	ND	43.02	13.11	ND	ND	ND	0.20	ND
Cake	18.00	ND	ND	39.99	ND	ND	ND	6.64	8.48	0.34

*ND - Not Detected

Humans can synthesize SFAs and MUFAs besides obtaining from the diet, while they cannot synthesize the parent PUFAs, namely, linoleic acid (LA, 18:2n-6) and alpha-linolenic acid (ALA, 18:3n-3). LA and ALA are dietary essential fatty acids, and are metabolized by consecutive chain elongase and desaturase enzymes to long chain (LC) n-6 PUFAs. Studies have shown that high intake of specific fatty acids (LA, ALA) lower the risk of CVD and CVD events (Liu *et al.,* 2017; Melanson *et al.,* 2009). However in the present study, the content of both LA and ALA was very low compared to other fatty acids in the foods analyzed, indicating the unhealthy quality of fat being consumed.

Processed foods tend to be energy-dense and have high quantities of unhealthy fats (saturated and trans-fat beyond the recommended level), which increase the risk of DR-NCDs (Mozaffarian *et al.,* 2006; Mozaffarian and Clarke, 2009; Mozaffarian *et al.,* 2010). Recognizing the health consequences of over consuming unhealthy fat, the World Health Organization (WHO) has highlighted the need to eliminate industrially produced trans-fat from the food supply and limit consumption of saturated fat as part of its global action plan for prevention and control of DR-NCDs (WHO, 2013; Shauna *et al.,* 2014).The Government of India has highlighted the consumption of trans fat, mainly through bakery products and fried goods as a public health problem and has taken steps towards its regulation which were published in the Gazette of India (FSSAI, 2014). The FSSAI is in the process of notifying the limits of trans-fat in all edible vegetable oils and fats to be not more than 2 per cent by weight in a phased manner by 2022 (FSSAI, 2014).

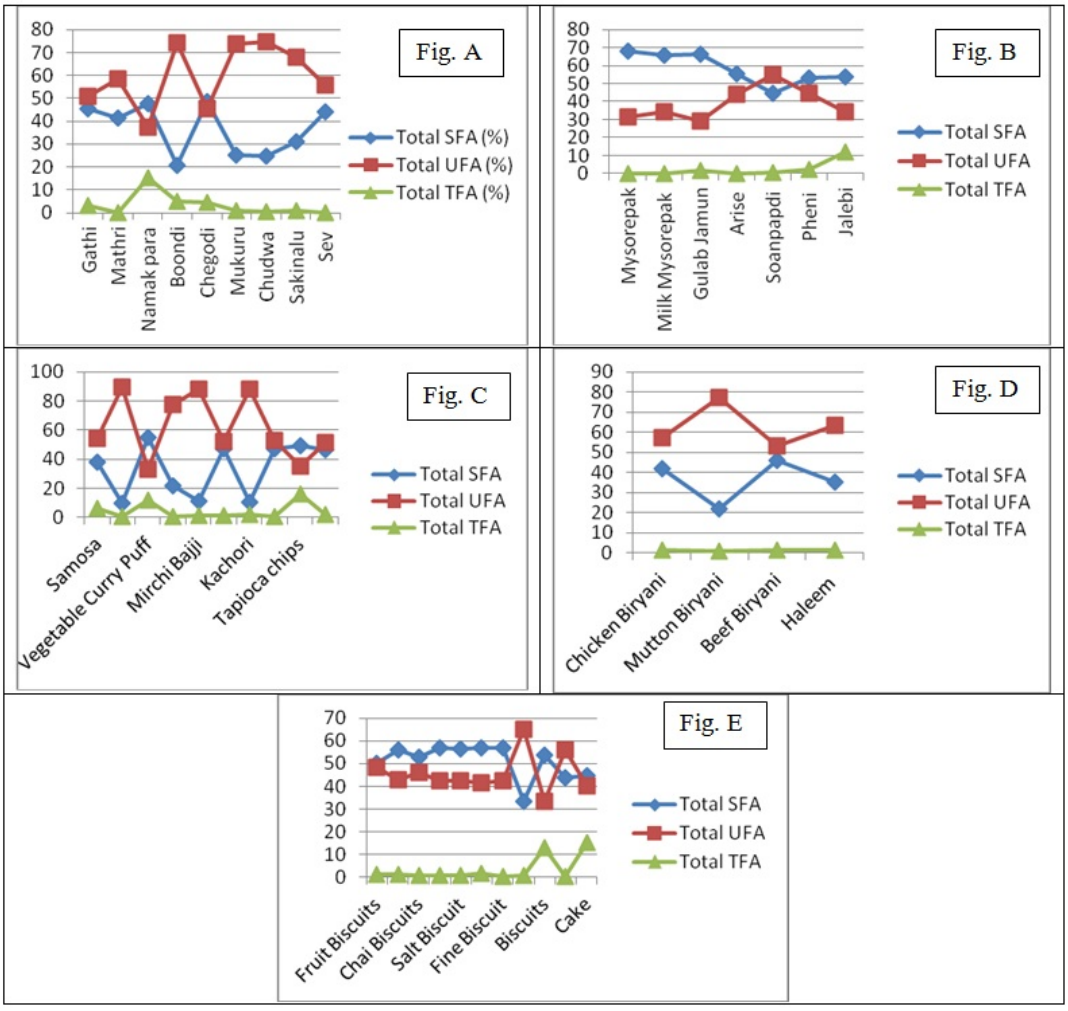

Fig. 1. Total fatty acid composition (SFA, UFA & TFA) of various foods without nutrition labels from unorganized sector (A: Total Fatty Acid Composition of Salted snack foods; B: Total Fatty Acid Composition of Sweets; C: Total Fatty Acid Composition of Street Foods; D: Total Fatty Acid Composition of Meal Items; E: Total Fatty Acid Composition of Baked Foods)

In the present study, trans fatty acids (TFA) Elaidic acid (C18:1, n9trans), Linolelidic acid (C18:2, n6trans) and Vaccenic acid (C18:1, n11trans) were analyzed in all the food samples. 92.7% of foods analysed had trans fat content in them. The TFA content varied between 0 - 8.10% in salted snack foods; 0 - 7.84% in sweets; 0 - 10.2% in street foods; 0 - 1.40% in meal items and 0 - 8.48% in baked foods. In spite of the policies initiated by FSSAI in India, presence of trans fats continues to be a hazard in most of the foods produced in the unorganized sector. The trans-fat content in Indian sweets and snacks has been estimated to range between 0.3 to 17.7 g/100 g and 0.1 to 19.8 g/100 g, respectively (Agrawal *et al.,* 2008). Other reasons for high consumption of SFA and TFA in India is due to consumption of fast foods and street foods purchased from independent street vendors (L'Abbé *et al.,* 2009; Ghafoorunissa, 2008). Palm oil and Vanaspati are used extensively by street vendors, because they remain stable after repeated heating and repeated heating of these fats can increase the quantity of trans-fat in the processed foods. Another problematic factor is that some vendors purchase used oil from larger restaurants, hotels, or caterers and use that oil for their food preparation (Downs *et al.,* 2013). All these factors continue to be contributing factors for consumption of poor quality fats as the vendors from unorganized sector purchase input ingredients that are affordable to them, but unhealthy to the consumer.

Apart from the above factors, all these foods do not have nutritional label and the consumer is not aware of the risk associated with the type of fat content in them. In spite of Food Safety and Standards - Packaging and Labelling Regulations 2011 (FSSAI, 2011) in place, there is little enforcement of labeling rules, especially for the processed, ready to eat foods and street foods from unorganized sector. Lack of consumer awareness regarding quality of fat, saturated fatty acids and trans-fat has the potential to limit the effectiveness of policies aimed at reducing their consumption, and continue to be available to the consumer. Despite the steady expansion of organized retailers, the unorganized sector vendors know that the consumer prefers purchase of several ready to eat processed foods from them, for various reasons (Singh., 2007). In this context, adopting a labeling system that is easier to interpret may increase the effectiveness of nutritional information (Blewett *et al.,* 2011; Kelly *et al.,* 2009) and could potentially cater to the Indian population with low literacy rates, by providing important information.

Over the past 50 years, dietary fat recommendations have shifted from total fat to type of fat. While consumption of UFA and SFA is important for inherent metabolic activities in the body, intake of higher levels than recommended is unwarranted. Consumption of ready to eat processed foods from unorganized sector can lead to consumption of higher amount of SFA and TFA beyond prescribed limits, which in turn can lead to various DR-NCDs. The results of the study indicate that street foods and baked foods had highest amount of SFA (44.79 - 68.08% of Total Fat), (33.49 - 57.05% of Total Fat) and TFA (0.68 - 15.90% of Total Fat), (0.20-15.46% of Total Fat) respectively, indicating consumption with great caution. Salted snack foods and sweets also had high amount of TFA (0.00-15.13% of Total Fat) and (0.00 - 11.77% of Total Fat). However, salted snack foods seem to be a better option among the two based on the results obtained. High amount of Palmitic acid (C16:0) and traces of Elaidic acid (C18:1, n9trans), Linolelidic acid (C18:2, n6trans) and Vaccenic acid (C18:1, n11trans) were the fatty acids found in most of the foods analysed. As there are no nutritional labels on all the foods from unorganized sector, consumers were largely unaware of the health implications of consuming high amounts saturated and trans-fatty acids. A greater transparency in labeling is needed through active consumer education to reduce the health risks associated with consumption of unhealthy fats. There is a need for more such information through regular surveillance on fatty acids content in processed foods from unorganized sector in India to support public health goals to ensure that the fat quality in processed ready to eat foods is improved.

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Diverse sources of resistance to different biotic stresses in elite groundnut (*Arachis hypogaea* L.) genotypes

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ABSTRACT

Groundnut, an important oilseed crop which is affected by many biotic and abiotic stresses. Among the biotic stresses, late leaf spot (LLS), rust and *Spodoptera litura*, are important in terms of their effect on reduction in yield. In the present study, forty-four elite genotypes of groundnut were assessed for their reaction to *Spodoptera litura*, late leaf spot and rust at a hot spot location. Three genotypes *viz*., DSG 1 (Virginia runner), Dh 216 (Spanish bunch) and ICGV 93468 (Virginia bunch) were resistant to *Spodoptera litura* having less than 10% leaf damage whereas, six genotypes *viz*., MG 8 dwarf, ICGV 05155, ICGV 87165, ICGV 87846, ICGV 06146 and JG (thin shell) were resistant to late leaf spot with <4 field disease score and nine genotypes were promising to rust by scoring <4 field disease score. There was a negative correlation between resistance to different stresses and maturity or productivity parameters. These genotypes belong to different botanical varieties of groundnut and hence can be effectively used for incorporation of resistance into varieties of specific botanical group. Among all the elite genotypes, ICGV 06146 had significantly higher pod yield/plant (23.2g) in addition to exhibiting resistance to late leaf spot and rust.

Keywords: Groundnut, Late leaf spot, Resistance, Rust, *Spodoptera litura*

Groundnut is one of the important oilseed crops in India. It is an invaluable source of oil, protein (Upadhyaya *et al.,* 2014), calories, essential fatty acids, vitamins, and minerals for human nutrition (Willett *et al.,* 2019). Though the crop has more production potential, the actual yields are very low which could be attributed to incidence of insects and diseases. Among the insects, tobacco cutworm, *Spodoptera litura* (Amin, 1983) and among diseases, late leaf spot and rust are the major ones reducing the groundnut yields drastically especially when the crop is grown under rainfed conditions during rainy season (Sandhikar *et al.,* 1989; Kolekar *et al.,* 2018).

Spodoptera litura (F.) is a pest of national importance and yield losses are reported to be 13-71% in the states of Karnataka and Andhra Pradesh (Amin, 1983). In India, transitional tract of Karnataka (Dharwad) has been identified as a hot spot for *S. litura* during rainy season, where yield loss to the extent of 66.6percent has been reported in groundnut (Kulkarni, 1989).

Among the foliar diseases, late leaf spot and rust were more threatening and pod loss of 50-80 per cent due to rust has been reported in an epidemic year (Sandhikar *et al.,* 1989). Late leaf spot can cause a reduction of 10-50 percent in yield (McDonald *et al.,* 1985). In transitional tract of Karnataka, late leaf spot resulted in yield loss up to 50 per cent (Puranik *et al.,* 1973; Astaputre and Kulkarni, 1996). Late leaf spot and rust occur together commonly whenever the groundnut is cultivated, but their incidence and damage level is different with respect to location and season. Both the diseases can individually cause economic level of yield loss. In India, it has been reported that late leaf spot and rust occur together and cause yield loss up to 70% (Subrahmanyam *et al.,* 1990).

Many chemicals can effectively control Spodoptera, late leaf spot and rust, but they are not eco-friendly and also add to the cost of cultivation. Under these circumstances, genetic resistance is the most desirable, economic and eco-friendly strategy to manage insects and diseases. This approach is gaining importance because of the narrow genetic variability present in the present-day cultivars which makes them easily susceptible to insect pests and diseases (Kumari *et al.,* 2014). Several genotypes resistant to late leaf spot and rust have been identified, but most of them are Valencia landraces and Virginia inter-specific derivatives with many undesirable features making them unsuitable for direct cultivation (Gowda *et al.,* 1995). In contrast, wild *Arachis* species have shown variation ranging from immune to highly resistance reaction to Late leaf spot and rust (Abdou *et al.,* 1974; Subrahmanyam *et al.,* 1985). However, the use of wild species in resistance breeding programs remained limited due to cross-compatibility barriers, occurrence of linkage drag, late maturity and undesirable pod and seed features. In this context, the present study is aimed at identifying the diverse sources of resistance to *S. litura,* late leaf spot and rust, from among elite genotypes consisting of advanced breeding lines and released cultivars belonging to different botanical groups of groundnut.

MATERIALS AND METHODS

Material consisted of forty-four elite genotypes comprising advanced breeding lines and released cultivars

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which were collected from ICRISAT, Hyderabad; BARC, Mumbai; UAS, Dharwad and UAS, Raichur. The list of these elite genotypes along with pedigree details is provided in Table 1. These genotypes were sown during 2017 rainy season at the hot spot location *viz.,* Main Agriculture Research Station, University of Agricultural Sciences, Dharwad (15° 13' N, 75° 07' E, 678 m above MSL, and 800 mm average annual rainfall). Each genotype was sown in 2 rows having a row length of 2 m in 2 replications under randomized complete block design (RCBD) which consist of 4 blocks. A spacing of 30 × 10 cm was followed for bunch genotypes and 60 × 10 cm for runner genotypes. After every five rows, one row of susceptible check JL 24 was sown to assure maximum incidence of the biotic stresses. Normal agronomic practices were followed to raise the crop avoiding plant protection measures.

Visual observations were made on per cent leaf damage due to *S. litura* (0-100 %) at 70 days after sowing (peak incidence period) by following the standard scale (0-9) (AICRP on Groundnut, 2015). The observation on percent leaf damage was assessed by leaf damage at top, middle and bottom leaves from five plants showing maximum damage due to insect in each genotype and expressed as mean percent leaf damage. Evaluation of genotypes against LLS and rust was undertaken at 80 days after sowing (DAS) which was coinciding with high incidence of both LLS and rust. The five plants selected in each genotype based on highest disease incidence were assessed for damage due to late leaf spot and rust by following the modified 9-point scale (Subrahamanyam *et al.,* 1995). Morphological and productivity parameters like height of the main stem, number of primary branches/plant, number of pods/plant, pod yield/plant, shelling percent and hundred seed weight were taken at/after harvest.

Analysis of variance and different components of genetic variation (PCV, GCV, H and GA) was performed for randomized complete block design (RCBD) in Windostat 9.1 version. Genotypic and phenotypic correlations were calculated to determine the direction and magnitude of association between resistance to Spodoptera, late leaf spot, rust and other productivity parameters and tested against table 't' values at n-2 degree of freedom both at 0.05 and 0.01 probability levels for their significance.

RESULTS AND DISCUSSION

Analysis of variance for reaction to different biotic stresses (*Spodoptera litura*, late leaf spot and rust), morphological (days to initiation of flowering, days to 50 percent flowering, plant height, number of primary branches) and productivity parameters (number of pods/plant, shelling percent, hundred seed weight and pod yield/plant) indicated highly significant genotypic differences for these traits (Table 2) which is essential for genetic improvement through plant breeding.

The difference between the phenotypic and genotypic coefficient of variation was very low for reaction to different biotic stresses, morphological and productivity parameters (Table 3) indicating less influence of environment component governing these traits. The extent of genotypic variability was high for response to *S. litura,* reaction to late leaf spot, rust and pod yield/plant indicating scope for selection of resistant genotypes against *S. litura,* late leaf spot and rust and also for pod yield in this material (Table 3). High heritability coupled with high genetic advance for response to *S. litura,* late leaf spot and rust and for number of pods/plant, hundred seed weight and yield/plant among the productivity parameters in elite genotypes (Table 3) revealed relatively higher additive component of genetic variance and hence genetic improvement for these traits would be possible through simple selection based on phenotype (Painwadee *et al.,* 2009). Earlier, Gangadhar *et al.* (2016) also reported high heritability coupled with high genetic advance for *S. litura* damage, late leaf spot and rust in groundnut. Higher additive component of genetic variation was also reported for late leaf spot, rust, number of pods/plant and yield/plant (Apte *et al.,* 2008; Raut *et al.,* 2010; Rao *et al.,* 2012).

There was a significant positive correlation between *S. litura* damage and late leaf spot incidence (Table 4) indicating that genotypes susceptible to *S. litura* were also susceptible to late leaf spot. Prasad (1997) also reported positive association between *S. litura* damage and late leaf spot incidence while studying groundnut mutants. *S. litura* damage had a significant negative correlation with days to initiation of flowering and days to fifty per cent flowering (Table 4) revealing that majority of the resistant genotypes were late in flowering and maturity. Majority of interspecific derivatives showing resistance to biotic stresses matured late (Naidu *et al.,* 2016).

S. litura damage also had significant negative correlation with yield/plant indicating resistant genotypes are poor yielders. This could be due to the fact that, during the development of cultivars, importance was given only towards increasing pod yield rather than resistance/tolerance to biotic stresses. Motagi *et al.* (1997) reported negative association of late leaf spot with yield in Spanish bunch mutants. This suggests the necessity to break negative associations through hybridization of induced mutations followed by selection.

There was a significant positive association between late leaf spot and rust (Table 4) which could be due to similar host pathogen interactions for late leaf spot and rust pathogens. Earlier, significant positive correlation was reported between late leaf spot and rust in studying diverse groundnut germplasm (Naidu, 2002) and mutants (Motagi, 2001).

Table 1 List of elite genotypes used in evaluation against different biotic stresses

Genotype	Pedigree	Source	Botanical group
TMV 2	Mass selection from 'Gudhiatham bunch'	UASD	SB
JL 24	Selection from EC-94943	UASD	SB
GPBD 4	KRG 1 × ICGV 86855	UASD	SB
GPBD 5	TG 49 × GPBD 4	UASD	SB
G2-52	Mutant of GPBD 4	UASD	SB
Dh 86	Dh 40 × Dh 8	UASD	SB
Dh 40	Dh 3-30 × TGE 2	UASD	SB
Mutant III	Mutant of VL 1	UASD	SB
TGLPS 3	TAG 24 × TG19	UASD	SB
Dh 3-30	Spanish improved × US 4	UASD	SB
DSG 1	Selection from Mardur local	UASD	VR
S 230	Selection from Tandur local	UASD	VB
JSP 39	JSP14 × JSSP-4	UASD	VB
Dh 8	Selection from RS 144	UASD	SB
Dh 216	TAG 24 × TGLPS 3-13	UASD	SB
Dh 101	R 9214 × Dh 51-2	UASD	SB
Dh 2000-1	JL 24 × ICGV 87165	UASD	SB
ICGV 06040	[(ICGV 92069 × ICGV 93184) × (NC Ac 343 × ICGV 86187)S23] F2-SSD-SSD-P5-B1-B1-B1	ICRISAT	VB
ICGV 06099	[(ICGV 92069 × ICGV 93184) × (NC Ac 343 × ICGV 86187)S23] F2-SSD-SSD-P5-P1-B2-B1	ICRISAT	VB
ICGV 06420	(ICGV 87846 × ICGV 99240) F2-P1-B1-B1-B1-B1-B1-B1-B1-B3	ICRISAT	VB
ICGV 05155	ICGV 99160 × ICGV 99240	ICRISAT	VB
ICGV 02266	(ICGV 94143 × ICGV 94136) F2-B1-B1-B1-B1-B1	ICRISAT	VB
ICGV 06146	[(ICGV 92069 × ICGV 93184) × (ICGV 96246 × 92 R/75)]	ICRISAT	VB
ICGV 91114	ICGV 86055 × ICGV 86533	ICRISAT	SB
ICGV 00350	ICGV 87290 × ICGV 87846	ICRISAT	VB
ICGV 87846	(CS 9 × ICGS 5) F2-B1-B2-B2-B1	ICRISAT	VB
ICGV 93468	ICGV 86015 × ICGV 86155	ICRISAT	VB
ICGV 86031	F334A-B-14 × NC Ac 2214	ICRISAT	SB
TAG 24	TGS-2 × TGE-1	BARCM	SB
TG 26	BARCG-l×TG-23	BARCM	SB
TG 37A	TG 25 × TG 26	BARCM	SB
TG 38	Girnar 1 × TG 26	BARCM	SB
TG 51	TG 26 × Chico	BARCM	SB
TG 67	TG 37A × CO 3	BARCM	SB
TG 68	TG 37A × Mutant 28-2	BARCM	SB
TG 69	Mutant of TG 66	BARCM	SB
TG 72	Mutant of TG 38	BARCM	SB
A30b	KRG 1 × ICGV 87165	UASD	VB
JG (Thin shell)	Not Available	UASD	SB
MG 8 (Dwarf)	Not Available	UASD	SB
ICGV 87165	PI 261942 × CS 9	ICRISAT	VB
R 8808	ICGS 11 × Chico	UASR	SB
R 9227	(ICGS 7 × NC Ac 2214) × ICGV 86031	UASR	SB
TKG 19A	TG 17 × TG 1	KKVD	SB

UASD-University of Agricultural Sciences, Dharwad; UASR-University of Agricultural Sciences, Raichur; KKVD-Konkan Krishi Vidyapeeth, Dapoli; ICRISAT-International Crop Research Institute for Semi Arid Tropics, Hyderabad; SB-Spanish Bunch; VB-Virginia Bunch; VR-Virginia Runner
BARCM-Bhaba Atomic Research Centre, Mumbai

Table 2 Mean sum of squares for various biotic stresses and productivity parameters among elite genotypes of groundnut

Phenotypic traits	Source of variation		
	Replication	Genotype	Error
df	1	43	43
Leaf damage by Spodopteralitura at 70 DAS	9.26	172.76**	3.48
Late leaf spot at 80 DAS	0.28	5.04**	0.21
Rust at 80 DAS	0.72	6.52**	0.30
Days to initiation of flowering	0.18	2.60**	0.36
Days to 50 % flowering	0.18	2.67**	0.39
Plant height (cm)	7.98	81.74**	6.60
Number of primary branches/plant	0.001	5.61**	0.21
Number of pods/plant	1.15	24.05**	4.09
Shelling/cent	0.88	18.80**	2.98
Hundred seed weight (g)	8.07	99.03**	3.27
Yield/plant (g)	2.24	25.80**	3.68

**- Significant at 1/cent level of probability

Table 3 Genetic components of variation for biotic stresses and productivity parameters among elite groundnut genotypes during *kharif* 2017

Traits / Components	Maximum	Minimum	Mean	PCV (%)	GCV (%)	Hbs	GA	GAM
Leaf damage by Spodoptera litura at 70 DAS	49.1	6.7	19.1	49.1	48.1	96.0	18.6	97.3
Late leaf spot at 80 DAS	8.0	2.5	5.9	27.3	26.3	91.9	3.1	51.8
Rust at 80 DAS	7.5	2.0	5.6	32.8	31.3	91.0	3.5	61.4
Days to initiation of flowering	33.0	27.0	29.5	4.1	3.6	75.2	1.9	6.4
Days to 50 % flowering	35.0	29.0	31.5	3.9	3.4	74.4	1.9	6.0
Plant height (cm)	47.7	20.6	29.3	22.6	20.9	85.0	11.6	39.7
Number of primary branches/plant	13.6	4.1	5.7	29.6	28.8	92.8	3.3	57.1
Number of pods/plant	25.2	11.2	17.4	21.6	18.2	70.8	5.5	31.5
Shelling/cent	78.6	66.7	73.3	4.5	3.8	72.6	4.9	6.7
Hundred seed weight (g)	63.1	25.9	40.9	17.5	16.9	93.6	13.8	33.7
Yield/plant (g)	23.5	11.4	17.1	22.4	19.7	77.5	6.12	35.8

PCV- Phenotypic co-efficient of variation (%); GCV- Genotypic co-efficient of variation (%); GA- Genetic advance; GAM- Genetic advance as/cent of mean; Hbs- Heritability (Broad sense)

Among the genotypes, one released cultivar DSG 1 and two advanced breeding lines ICGV 93468 and Dh 216 showed resistance to *S. litura* with less than 10 per cent leaf damage (Table 5). DSG 1 is a Virginia runner genotype and ICGV 93468 is a Virginia bunch genotype, took more days to 50% flowering while, Dh 216 is a Spanish bunch genotype with less days to 50% flowering. These genotypes were susceptible to late leaf spot and rust. Earlier, ICGV 86031 was reported as resistant to multiple biotic stresses including *Spodoptera* (Dwivedi *et al.,* 1993). But, this genotype in the present study showed 14.1 per cent leaf damage (data not presented). In another study, highest resistance to *Spodoptera* was reported in three Virginia runner genotypes, NC Ac 17840, NFG 79 and EC 21989 (Rajagopal *et al.,* 1988). The genotypes Dh 216, ICGV 93468 and DSG 1 can be used in breeding programme for incorporation of Spodoptera resistance into Spanish bunch, Virginia bunch and Virginia runner cultivars, respectively. The genotype ICGV 93468 had significantly superior pods/plant (19.2) compared to JL 24 (14.2).

In case of late leaf spot, only six genotypes *viz.,* MG 8 dwarf, ICGV 05155, ICGV 87165, ICGV 87846, ICGV 06146 and JG (thin shell) had resistance with less than 4 field disease score (Table 6). Among these genotypes, ICGV 05155, ICGV 87165, ICGV 87846 and ICGV 06146 were also found promising to rust. None of them were resistant to *Spodoptera.* Earlier, ICGV 06146 (Rani *et al.,* 2018) and ICGV 87165 (Moss *et al.,* 1997; Motagi *et al.,* 2014; Rani *et al.,* 2018) were reported to be resistant to late leaf spot. ICGV 87165, a Virginia bunch interspecific derivative was identified as resistant to late leaf spot, rust (Motagi *et al.,* 2014) bacterial wilt, leaf miner and *S. litura* (Moss *et al.,* 1997). In the present study, this genotype was having higher leaf damage due to *Spodoptera* (19%) (Table 6) but had significantly higher pods/plant (21.9). Whereas three genotypes, ICGV 87846, MG 8 and JG (thin shell) had

significantly higher hundred seed weight (56.3 g, 48.7 g and 46.9 g, respectively) and can be used for incorporation of late leaf spot resistance in confectionary groundnut. Among all the resistant genotypes, ICGV 06146 was promising to rust besides having significantly higher pod yield/plant (23.2 g) and hence can be used in incorporation of resistance to both late leaf spot and rust.

There was less rust incidence during the season that was evident with a field disease score of 6.5 (on a 1 to 9 scale) in the susceptible check, JL 24. This was due to predominance of late leaf spot over rust wherein late leaf spot disease incidence could have started early in the season compared to rust. Among the 44 genotypes, nine genotypes *viz.,* ICGV 87846, ICGV 06146, ICGV 05155, ICGV 02266, G 2-52, A30b, GPBD 4, GPBD 5 and ICGV 87165 appeared to be promising for rust resistance with less than 4.0 field disease score, compared to 6.5 score in susceptible check JL 24 (Table 7). Earlier, ICGV 05155 and ICGV 87846 (Chaudhari *et al.,* 2019) were reported as resistant to rust, and ICGV 06146 was reported as resistant to foliar diseases and moderately resistant to collar rot (Rani *et al.,* 2018). Among promising rust resistant genotypes, ICGV 87846, ICGV 06146, GPBD 4, ICGV 87165 and ICGV 05155 had also resistance to late leaf spot (<4) and ICGV 87846 had significantly large seed size (56.3g/100 kernels).

Table 4 Phenotypic and genotypic correlations among various biotic stresses and productivity parameters in groundnut elite genotypes

Traits	Spodoptera damage	Late leaf spot	Rust	Days to initiation of flowering	Days to 50 % flowering	Plant height	No. of primary branches/plant	No. of pods/plant	Shelling/cent	Hundred seed weight	Yield/plant
Spodoptera damage	1.000	0.239*	0.106	-0.284**	-0.330**	0.400**	-0.034	-0.141	-0.057	-0.145	-0.282**
Late leaf spot	0.259*	1.000	0.620**	-0.235*	-0.169	-0.038	-0.175	0.159	-0.134	-0.169	-0.133
Rust	0.112	0.700**	1.000	-0.201	-0.149	-0.143	-0.350**	-0.192	-0.293**	-0.222*	0.027
Days to initiation of flowering	-0.348**	-0.237*	0.261*	1.000	0.803**	0.007	0.140	-0.052	-0.013	0.130	-0.050
Days to 50 % flowering	-0.394**	-0.201	-0.220*	0.872**	1.000	-0.033	0.137	-0.032	-0.066	0.212*	-0.080
Plant height	0.414**	-0.066	-0.153	-0.014	-0.065	1.000	0.025	-0.039	0.187	-0.121	-0.294**
No. of primary branches/plant	-0.024	-0.193	-0.365**	0.184	0.138	0.058	1.000	0.116	0.009	-0.055	-0.090
No. of pods/plant	-0.220*	-0.228*	-0.246*	-0.045	-0.040	-0.146	0.1593	1.000	0.055	-0.128	0.079
Shelling/cent	-0.0717	-0.121	-0.367**	-0.111	-0.087	0.185	0.087	0.019	1.000	0.315**	0.039
Hundred seed weight	-0.142	-0.202	-0.234*	0.207	0.256**	-0.114	-0.065	-0.182	0.387**	1.000	0.022
Yield/plant	-0.336**	-0.165	-0.020	-0.058	-0.125	-0.319**	-0.113	0.196	0.104	0.017	1.000

*& **- Significant at 5 and 1/cent level of probability, respectively

Values above the diagonal represent phenotypic correlation while the below diagonal represent genotypic correlation

Table 5 Mean performance of elite groundnut genotypes showing <10/cent *Spodoptera litura* damage for foliar diseases and productivity traits

Genotypes	*Spodoptera* damage (%)	Late leaf spot	Rust	Days to initiation of flowering	Days to 50 % flowering	Plant height (cm)	Number of primary branches/plant	No. of pods/plant	Shelling/cent	Hundred seed weight (g)	Yield/plant (g)
DSG 1	6.8	5.5	6.5	31.5**	33.5**	45.0**	6.0**	15.2	71.1	36.4	11.4
Dh 216	7.0	7.5	7.0	28.0	30.5	22.9	5.2	16.0	76.0	35.2	19.8
ICGV 93468	9.5	7.0	7.0	29.5**	31.5**	28.8	5.7*	19.9**	66.8	41.5	16.7
Checks											
ICGV 86031	14.1	7.0	7.0	28.5	31.0*	31.0	4.4	18.0	72.6	34.2	21.6
JL 24	45.7	7.5	6.5	27.0	29.5	42.9	5.8	14.2	74.4	41.4	15.6
Mean	19.1	5.9	5.6	29.5	1.5	29.3	5.7	17.4	73.6	40.9	17.1
C.D. (5%)	3.7	0.9	1.1	1.2	1.3	5.2	0.9	4.1	3.5	3.6	3.9
C.D. (1%)	5.0	1.2	1.5	1.6	1.7	6.9	1.2	5.5	4.7	4.9	5.2
C.V. (%)	9.8	7.8	9.9	2.1	2	8.8	8.0	11.6	2.3	4.4	11.2

*&** - indicates the superiority of the genotype over the genotype JL 24 at 5/cent and 1/cent level of probability, respectively.

Table 6 Mean performance of elite groundnut genotypes showing < 4 score to late leaf spot for rust, Spodoptera and productivity traits

Genotypes	Late leaf spot	Rust	Spodoptera leaf damage (%)	Days to initiation of flowering	Days to 50 % flowering	Plant height (cm)	Number of primary branches/plant	No. of pods/plant	Shelling/cent	Hundred seed weight (g)	Yield/plant (g)
MG 8	2.5	5.5	17.8**	29.5**	31.5**	28.9	4.4	15.8	72.5	48.7**	12.7
ICGV 05155	2.5	2.5	22.0**	30.0**	31.5**	32.4	5.4	16.4	69.5	38.1	19.7
ICGV 87165	3.0	4.0	19.0**	29.5**	32.0**	34.4	5.3	21.9**	74.8	36.9	12.2
ICGV 87846	4.0	2.0	12.2**	29.5**	31.5**	22.5	7.0**	14.1	76.9	56.3**	19.6
JG (Thin shell)	4.0	5.5	12.2**	29.5**	32.0**	25.1	4.3	13.2	76.3	46.9*	16.0
ICGV 06146	4.0	2.0	20.2**	29.5**	31.5**	29.5	6.4	17.7	73.4	42.8	23.2**
Checks											
GPBD 4 (R)	4.0	3.0	25.7	28.0	29.5	45.0	7.8	17.9	76.3	37.3	16.4
JL 24 (S)	7.5	6.5	45.7	27.0	29.5	42.9	5.8	14.2	74.4	41.4	15.6
Mean	5.9	5.6	19.1	29.5	1.5	29.3	5.7	17.4	73.6	40.9	
CD (5 %)	0.9	1.1	3.7	1.2	1.3	5.2	0.9	4.1	3.5	3.6	
CD (1 %)	1.2	1.5	5.0	1.6	1.7	6.9	1.2	5.5	4.7	4.9	
CV (%)	7.8	9.9	9.8	2.1	2	8.8	8.0	11.6	2.3	4.4	

*&** - indicates the superiority of the genotype over the genotype JL 24 at 5/cent and 1/cent level of probability, respectively

Table 7 Mean performance of elite groundnut genotypes showing <3 field disease score to rust for late leaf spot, Spodoptera and productivity traits

Genotypes	Rust	Late leaf spot	*Spodoptera* damage (%)	Days to initiation of flowering	Days to 50 % flowering	Plant height (cm)	Number of primary branches/plant	No. of pods/plant	Shelling/cent	Hundred seed weight (g)	Yield/plant (g)
ICGV 87846	2.0	4.0	12.2	29.5	31.5	22.5	7.0	14.1	76.9	56.3**	19.6
ICGV 06146	2.0	4.0	20.2	29.5	31.5	29.5	6.4	17.7	73.4	42.8**	23.2**
G 2-52	2.0	5.0	14.9	33.0	35.0	34.0	5.7	14.6	74.4	37.4	12.1
ICGV 05155	2.5	2.5	22.0	30.0	31.5	32.4	5.4	16.4	69.5	38.1	19.7
A30b	2.5	4.5	15.0	29.5	32.0	25.7	13.6**	23.4**	74.6	35.9	14.5
GPBD 5	2.5	4.5	17.7	28.5	30.5	27.0	5.4	25.2**	77.7	40.4	16.8
ICGV 02266	3.0	4.5	14.5	30.0	32.0	30.5	5.1	21.5**	73.1	41.9*	22.9**
ICGV 06420	3.5	5.5	12.8	30.5	32.5	29.1	7.4	17.8	77.3	41.1	17.3
ICGV 87165	4.0	3.0	19.0	29.5	32.0	34.4	5.3	21.9**	74.8	36.9	12.2
Checks											
GPBD 4	3.0	4.0	25.7	28.0	29.5	45.0	7.8	17.9	76.3	37.3	16.4
JL 24	6.5	7.5	45.7	27.0	29.5	42.9	5.8	14.2	74.4	41.4	15.6
Mean	5.6	5.9	19.1	29.5	1.5	29.3	5.7	17.4	73.6	40.9	17.1
CD (5 %)	1.1	0.9	3.7	1.2	1.3	5.2	0.9	4.1	3.5	3.6	3.9
CD (1 %)	1.5	1.2	5.0	1.6	1.7	6.9	1.2	5.5	4.7	4.9	5.2
CV (%)	9.8	9.9	7.8	2.1	2	8.8	8.0	11.6	2.3	4.4	11.2

*&** - indicates the superiority of the genotype over the genotype JL 24 at 5/cent and 1/cent level of significance, respectively

The resistant genotypes identified in the present study need to be confirmed and utilized in future breeding programme as diverse sources of resistance. The genotype, ICGV 06146 was resistant to both late leaf spot and rust, in addition to having significantly higher pod yield (23.2 g/plant) and hence can be a potential genotype for incorporation of foliar disease resistance. This genotype was earlier identified as resistant to late leaf spot and rust and also moderately resistant to collar rot (Rani *et al.,* 2018) and iron deficiency chlorosis (Boodi *et al.,* 2015). Hence, this genotype can be used as a potential multiple stress resistant genotype in groundnut breeding programme. Thus, the present study has identified diverse resistant sources for individual biotic stresses and also multiple biotic stresses.

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Studies on transmission of phyllody from sesame to alternate host periwinkle (*Vinca rosea*)

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ABSTRACT

Phyllody is a serious disease of sesame worldwide and the Present investigation was carried out on transmission of this disease to periwinkle. Infected plants showed characteristic symptoms of witches' broom, stunting, leaf proliferation and bushy appearance. Under field condition, infected plants showed the most common characteristic phyllody symptoms. The pathogen phytoplasma could be successfully transmitted through grafting, leafhopper and dodder from infected sesame to healthy periwinkle plants. Among the methods, leafhopper vector (*Orosius albicinctus*) mediated transmission of phytoplasma was more efficient from infected sesame to periwinkle plants. Plants raised from infected periwinkle seeds did not show any phyllody symptoms.

Keywords: Dodder, Leafhopper, Periwinkle, Phyllody, Phytoplasma, Sesame, Transmission

Sesame (*Sesamum indicum* L.) is one of the important oil seed crops grown in many parts of the world. It is also known as queen of oil seeds. It is a rich source of lignins such as sesamin, sesamol and sesamoline (Shyu and Hwang, 2002) which are antioxidants that impart long shelf life to the oil by resisting the oxidation (Brar and Ahuja, 1979). The oil content varied between 41.3 and 62.7%, the average being 53.3% sesame seed is rich in oil, contains high amounts of unsaturated fatty acids (83-90%), mainly linoleic acid (37-47%), oleic acid (35-43%), palmitic (9-11%) and stearic acid (5-10%) with trace amount of linolenic acid (Pathak *et al.,* 2014). The sesame seeds are rich source of edible oil (50%), protein (20%), oleic acid (47%) and linolenic acid (39%). In India the area of sesame is 1.6 million ha with the production of 0.75 million tonnes and productivity of 478 kg/ha (Ministry of Agriculture, Government of India, 2018).

Sesame is described as the "Queen of oil seeds" because of its high oil content (50-60%), protein (18-25%), calcium, phosphorous, oxalic acid and it also has excellent qualities of the seed oil and meal (Prasad, 2002). The edible oil is used for cooking and also used in preparation of medicines and high quality soaps. Sesame cake is an excellent feed for cattle and layers (Khan and Shaik, 1985). Sesame oil also contains high level of unsaturated fatty acids,which has a reducing effect on the plasma cholesterol (Banerjee and Kole, 2006).

Sesame is a short duration crop and is grown mostly under rainfed conditions with poor crop management resulting in low yields. Pests and diseases are the other major factors for the reduction in yield. As many as 29 species of insect pests attack the crop at different stages of crop growth (Rai, 1976). Sesame is vulnerable to infection by a number of diseases *viz.,* charcoal rot/ root rot/ stem rot, bacterial leaf spot, leaf blight, powdery mildew, wilt, leaf spot, phyllody and stem blight that cause considerable yield losses. Among the major diseases, economically important diseases affecting sesame are phyllody, root/stem rot and powdery mildew (Karibasappa *et al.,* 2018). The sesame phyllody became epidemic in India, majorly in the peninsular regions with incidence of approximately 75% (Manjunatha *et al.,* 2012). The sesame phyllody causes a yield loss upto 80 % (Salehi *et al.,* 2017). The cause of disease was assumed to be a virus, which was later confirmed as mycoplasma like organisms (MLO) and termed as phytoplasmas (Das and Mitra, 1998). About one per cent increase in disease intensity decreases sesame yield by 8.36 kg/ha (Maiti *et al.,* 1988).

The phytoplasmas are specialized bacteria which do not have cell wall, non-helical, obligate, intracellular parasites belonging to the class mollicutes and reside in the phloem of the plants (Doi *et al.,* 1967). In cross section of affected plant parts, phytoplasma appear as rounded pleiomorphic bodies with an average diameter of 200 to 800 µm (Lee *et al*., 2000). The affected plants exhibit a wide range of symptoms like shoot proliferation, smaller leaves, virescense, abnormal floral organs, sterility of flowers, necrosis of phloem and plant decline (Bertaccini and Duduk, 2009) sometimes these symptoms are accompanied with yellowing, cracking of seed capsule, vivipary and formation of dark exudates on the foliage (Akhtar *et al.,* 2009). Phytoplasmas are transmitted by the phloem feeding insects such as planthoppers, psyllids and leafhoppers. In addition to the insect vectors, the disease could be transmitted by the

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parasitic dodder as well as grafting of infected material to healthy plant, but it is known not to be transmitted through sap and seed (Gogoi *et al.,* 2017). Limited information is available regarding transmission of phytoplasma to periwinkle. Therefore, the present investigation was carried out to assess different methods for transmission of this disease to periwinkle.

MATERIALS AND METHODS

Four methods of transmission, seed, grafting, insect mediated and dodder mediated, were tried. In each case at least 5 plants were used for transmission study. After the transmission attempt, number of plants showing symptoms and time taken for symptom development was observed.

Seed transmission: Matured seeds from phyllody infected periwinkle and healthy periwinkle plants were collected and stored in the laboratory. Twenty seeds each from diseased and healthy periwinkle plants were raised on earthen pots under net house conditions and observations were made with respect to plants exhibiting the symptoms.

Graft inoculation: Ten 4-week-old periwinkle plants were graft inoculated using phyllody disease scions under greenhouse conditions. Side grafting method was employed for graft transmission. A 13-cm long sesame branch exhibiting typical phyllody symptoms was detached from an infected plant and the stem was to give a 'V' shaped. end. This scion was inserted into the slanting cut of same shape made on the healthy root stock plant of periwinkle. The grafted portion was tied tightly with a parafilm. The inoculated plants were kept in insect proof net house for symptom development and observations were recorded over time.

Insect transmission: Wooden insect rearing cages measuring 45x45x30 cm^3 fixed with wire mesh were used for the studies. Healthy periwinkle plants were kept in the cages to transmit the phytoplasma from infected sesame to healthy periwinkle plants. An aspirator comprising a glass tube (10 cm length and 2 cm diameter) and a rubber tube of 15 cm length was used for the collection of nymphal leafhoppers. The nymphal leafhoppers were collected from sesame field by gently turning the leaves upwards and sucking with an aspirator during early morning and evening. The phyllody infected sesame was kept in the cage and through the narrow mouth of the aspirator the nymphs were released into the cage and then tightly closed. About 20-25 nymphal leafhoppers were collected from infected field and released into cages in which infected sesame plant were previously placed and these nymphs were allowed to feed for 15 days as acquisition feeding period. After the acquisition feeding period, the viruliferous leafhoppers were taken out and allowed to feed on healthy seedling. About 10-15 viruliferous nymphal leafhoppers collected from acquisition cages were released into inoculation cages with healthy periwinkle seedlings and allowed to feed for 15 days as an inoculation feeding period. The leafhoppers in the inoculation chambers were killed using insecticide after the inoculation feeding period was over. Inoculated plants were continuously monitored for symptom expression. Data on percent disease incidence, time required for appearance of the symptoms were recorded.

Dodder transmission: Dodder transmission study was made according to Marcone *et al.* (1997). Dodder (*Cuscuta campestris*) strands were established on phyllody infected periwinkle plants for four weeks. The newly developed dodder strands from diseased plants were then attached to healthy four week-old periwinkle seedlings (Fig. 4.4). The dodder bridge between the phyllody infected plant and the inoculated plants were detached after four weeks and observed regularly for symptom development. Observations were recorded on number of plants showing symptoms and time taken for symptom development.

Molecular confirmation with PCR: Periwinkle plants showing typical symptoms of phyllody and healthy periwinkle plants used as a control were collected from pot culture studies. DNA was isolated from infected and healthy leaf samples by using modified CTAB method (Sunard *et al.,* 1991). The isolated DNA samples were stored at -20ºC for further use.

Isolated genomic DNA was used as a temFig. in first round PCR for amplification of 16SrRNA of phytoplasma using P1/P7 primers (Deng and Hiruki, 1991; Smart *et al.,* 1996) followed by nested PCR using 2 µl of diluted first step PCR product with phytoplasma specific nested primers R16F2n/R16R2 (Gundersen and Lee 1996). The direct PCR and nested PCR were carried out sequentially in a final volume of 20 µl reactions containing 2.0 µl of 10X PCR buffer with $MgCl_2$, 2.0 µl of 2.5 mM dNTP mixture, 1.0 µl (5 pM) of each primers, 0.5 µl Taq DNA polymerase (3 U/ µl), and 2 µl temFig. DNA (50 ng/ µl). The DNA was amplified with a PCR profile of initial denaturation of 94°C for 2 min followed by 30 cycles of 94°C for 1 min denaturation, 52°C for 1 min primer annealing, 72°C for 2 min primer extension and final extension at 72°C for 5 min. The PCR products were analysed by electrophoresis in 3 % (w/v) agarose gel. The image of DNA fragments in the gel was captured using gel documentation system. The amplicon of nested PCR was sequenced and the obtained sequence was compared with the sequences available with NCBI using the standard BLAST analysis.

Fig. 1a. Side grafting of infected sesame scion on healthy periwinkle stock

Fig 1b. Transmission of phytoplasma through grafting- Yellowing symptom on graft inoculated periwinkle plant

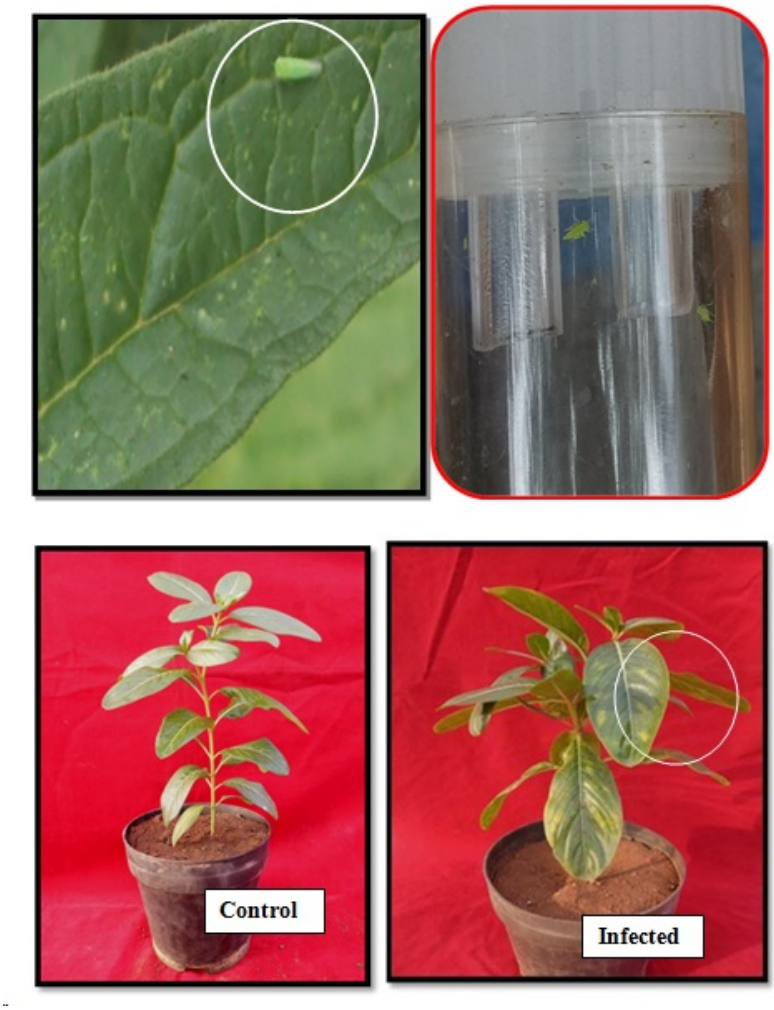


Fig. 2 Transmission of Phytoplasma through leafhopper

Fig. 3a. Establishing Cuscuta on periwinkle

Fig. 3b. Dodder transmission of phyllody to periwinkle

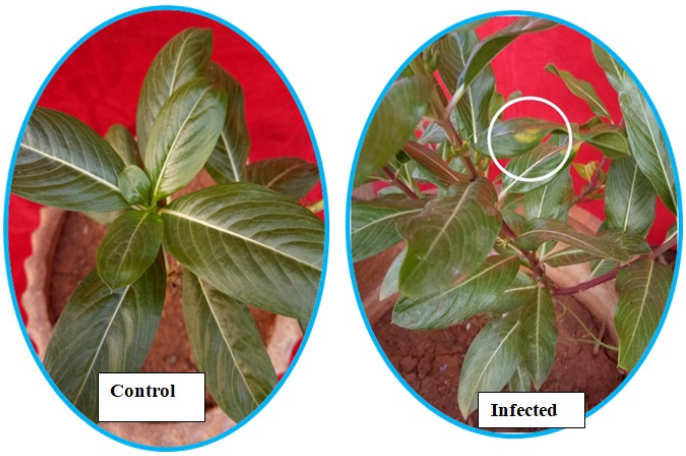
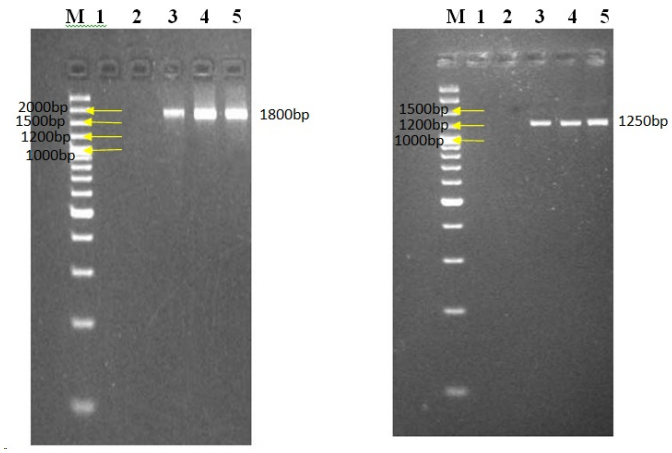


Fig. 3c. Yellowing symptom on dodder inoculated periwinkle plant



Direct PCR Nested PCR

Fig. 4. Confirmation with PCR

M- 100 bp plus marker
1-Negative control (Healthy plant sample)
2-Phytoplasma transmitted through seed
3- dodder inoculated Periwinkle
4- graft inoculated Periwinkle
5- leafhopper inoculated Periwinkle

RESULTS AND DISCUSSION

Seed transmission: Seed transmission of the infectious agent could not be achieved under greenhouse conditions, which indicated that phyllody disease was not seed transmissible. This result is in agreement with earlier reports (Akhtar *et al*., 2009; Pathak *et al*., 2012).

Graft inoculation: The Phytoplasmas move within the plants through phloem from source to sink and they efficiently pass through sieve tube elements in phloem (Christensen *et al.,* 2004). This feature of phytoplasma existence and survival in the plants might facilitate the transmission of the phytoplasma through the process of grafting. Consistent with this hypothesis, the causative agent of sesame phyllody was successfully transmitted to four healthy periwinkle plants through grafting as observed by the development of characteristic symptoms of phyllody such as small leaves, yellowing of leaves and stunted growth in periwinkle (Fig. 1a and b) at 45-50 DAI and the infected plants showed presence of the phytoplasma as indicated by the PCR results (Fig. 4). The results clearly indicated that all the plants exhibiting symptoms had phytoplasma and thus suggested that grafting procedure ensured 100 per cent transmission rate of disease (Table 1). Similar observations have been reported earlier. Akhtar *et al*. (2009) reported transmission of sesame phytoplasma from diseased to healthy sesame by grafting and they observed symptoms within 25-35 DAI. Ravindar (2017) reported 85 per cent of phyllody disease transmitted into the graft inoculated plants and Ikten *et al*. (2014) observed characteristic symptoms of phyllody, floral virescence, yellowing of leaves and stunted growth when transmitted through graft inoculation.

Table 1 Transmission of sesame phyllody to periwinkle through grafting, leafhopper and dodder

Method of Transmission	Total no. of plants		Symptoms observed	Days taken for appearance of symptoms	% of infection
	Inoculated	Infected			
Grafting	5	4	Small leaves, yellowing of leaves and stunted growth	45-50	80
Leafhopper	10	10	Small leaves, yellowing of leaves and stunted growth	55-60	100
Dodder	5	4	Small leaves, yellowing of leaves and stunted growth	60-65	80

Grafting has been demonstrated as a method for transmission of phytoplasma diseases in other crops as well. Salehi *et al*. (2009) reported the successful transmission of safflower phyllody phytoplasma through grafting to healthy safflower and observed characteristic symptoms such as very small leaves, phyllody, floral virescence, yellowing of leaves and stunted growth within 45-70 DAI. Jarausch *et al*. (1999) observed successful transmission of European stone fruit yellows (ESFY) phytoplasma by graft transmission. Kamska and Korbin (1999) reported graft transmission of phytoplasma affecting lily plant.

Insect transmission: Phyllody disease was previously observed to be vectored by *O. albicinctus* in India (Kolte, 1985; Srinivasulu and Narayanasamy, 1995) and Iran (Esmailzadeh-Hosseini *et al.,* 2007), by *O. cellulosus* Lindberg in Upper Volta (Desmits and Laboucheix, 1974), and by *Neoaliturus haematoceps* forma opacipennis (*J. Dlabola*, pers. comm.) in Iran (Salehi and Izadpanah, 1992). Phyllody has also been transmitted from diseased sesame to *Catharanthus roseus* L. by *Circulifer haematoceps* in Turkey (Kersting, 1993). Therefore, it was hypothesized that leaf hoppers raised on phyllody affected sesame plants could transmit the disease to the healthy plants. Our results demonstrated that the sesame phyllody phytoplasma was successfully transmitted from infected sesame to healthy periwinkle plants (Fig. 2) by the leafhopper Orosius albicinctus. In periwinkle, out of the 10 leafhopper inoculated plants, all the inoculated plants showed phyllody symptoms at 55-60 DAI. The infected plants showed characteristic symptoms such as small leaves, yellowing of leaves and stunted growth (Fig. 2) and these plants exhibited positive reaction to the phyllody in both direct and nested PCR (Fig. 4). No symptom was observed in plants kept in control cage. These results clearly indicated that the leafhopper *O. albicinctus* successfully transmitted the phytoplasma from infected sesame plants to 100 per cent of healthy plants (Table 1). It has been earlier reported that *Orosius* spp. could transmit sesame phyllody to sun hemp, chickpea and berseem under natural conditions (Vasudeva and Sahambi, 1955; 1959). The results of transmission assay also confirmed that the leafhopper *O. albicinctus* plays a major role as a natural vector of phytoplasma associated with sesame phyllody disease and could be the main route of transmission under field conditions.

Dodder transmission: Dodder (*Cuscuta campestris*) successfully transmitted the phyllody from infected plants to healthy periwinkle plants under net house conditions (Fig. 3a). The water imbibed dodder seeds were sown in pots having phyllody infected sesame plants. The established dodder strands were twined on periwinkle plants and these plants were used for transmission of phytoplasma through dodder. In periwinkle out of the 5 inoculated plants, 4 inoculated plants exhibited the characteristic symptoms of phyllody such as small leaves, yellowing of leaves and stunted growth (Fig. 3a, b and c) at 60-65 DAI. While no symptoms were developed in the control plants. In PCR assay the infected plants showed positive reaction to the phyllody in both direct and nested PCR (Fig. 4). The results clearly indicated that the dodder (Cuscuta campestris) successfully transmitted the phytoplasma from infected plants to 80 per cent of healthy periwinkle plants (Table 1).

The results clearly indicated that dodder acts as an efficient means in natural transmission of the disease in the field. Earlier, Abraham *et al.* (1977) reported that dodder (*Cuscuta campestris*) played a role in transmission, and acted as donor as well as reservoir of sesame phyllody phytoplasma.

Similarly, many other workers have reported successful transmission of the phyllody disease from infected sesame to healthy sesame with the help of dodder (Salehi and Izadpanah, 1992; Akhtar *et al.,* 2009; Pathak *et al.,* 2012). While Marcone *et al.* (1997) noticed successful dodder transmission of alder yellows phytoplasma to the experimental host *Vinca rosea* (periwinkle). Similarly, Salehi *et al.* (2009) demonstrated the successful transmission of safflower phyllody phytoplasma through dodder.

Sequencing of phytoplasma 16S rDNA: 16S rDNA from periwinkle samples were collected and amplified by PCR using 16S rDNA specific primers P1/P7 and R16F2n/R16R2 and obtained 1250 bp product in all isolates transmitted through grafting, leafhopper and dodder (Fig. 4.4). The 1250 bp product was sequenced and compared with those of phytoplasma species accessible in the GenBank using BLAST similarity search tool. The sequence obtained in this study was found to be 100 % similar to the members of the 16S rII group, Candidatus Phytoplasma aurantifolia, that contains phytoplasmas associated with sesame phyllody from South India.

Confirmation of presence of phyllody in artificially infected plants: The phytoplasma infected periwinkle samples showed production of little leaves, yellowing, stunted growth and phyllody. DNA was isolated from phytoplasma infected periwinkle samples by CTAB method. The amount of DNA and purity of DNA (260/280 ratio) was measured in Nanodrop spectrophotometer. This DNA was used as temFig. in direct and nested PCR with universal primers P1/P7 and R16F2n/R16R2.

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Trade dynamics and export supply function of Indian groundnut

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ABSTRACT

India is the leading groundnut exporter to world market. Direction of trade has changed over the period. Sphere of groundnut export has narrowed down from European nations during 1995-96 to Asian markets during 2014-15, with lost shares in UK, Netherland, Egypt, USA and Russia to Vietnam, Thailand, Pakistan, China, Ukraine and UAE. Indonesia was identified as highest loyal market followed by Malaysia. To identify the factors influencing groundnut export, Cointegration and Vector Error Correction Model were used. Export was found to increase significantly with export price and exchange rate and decrease significantly with increase in domestic price and increase in population. Speed of adjustment indicates the adjustment to the long-run equilibrium from short run shocks. Result highlights to target European market, the largest importer of groundnut in world.

Keywords: Cointegration, Direction of trade, Groundnut export, Markov chain analysis, Vector Error Correction Model

India exports groundnut in different forms namely, shelled, in-shell and prepared groundnut but more than 90 per cent of total is exported in shelled or kernel form (http://agriexchange.apeda.gov.in). During 2017-18, India ranked second in groundnut export to world market with 5.22 lakh tonnes export (20.58%) next only to Argentina (5.66 lakh tonnes; 22.33%) followed by USA (4.41 lakh tonnes; 17.39%) and China (3.12 lakh tonnes; 12.31%). On the other hand, Netherlands, Indonesia, Germany, Mexico and Russia are the leading importers with 3.45, 2.87, 1.27, 1.65 and 1.32 lakh tonnes groundnut imports, respectively (APEDA, 2019). India exports groundnut to more than hundred countries in one or other years. But sphere of her export has narrowed down from European to Asian markets consequently direction has changed over the period. The export of an agricultural commodity depends on available surplus of that commodity along with other price and non-price factors. High and increasing domestic demand due to large and rising population and increasing income adversely affects India's agricultural exports (Ansari and Khan, 2015; Murlidhar Meena *et al.,* 2018). Understanding how area, production, domestic prices, international prices, exchange rates along with other socio-economic factors like GDP and population influence the groundnut export from India is imperative from a policy perspective. The study of change in direction of export will help identify major destinations and their prudence over the period. Study will help to decide whether the changes are in the desirable directions or if changes are needed to boost sales to a particular market. It will aid in articulating export promotion policies to strengthen the export performance of Indian groundnut in international market.

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MATERIALS AND METHODS

Data: Data on groundnut export from India (in quantity term) country-wise, Harmonized System code wise and the total, were collected from 1996-97 to 2014-15 from Directorate General of Commercial Intelligence and Statistics (DGCIS, Kolkata), available at APEDA website (http://agriexchange.apeda.gov.in). The information on area, production and productivity was collected from Directorate of Economics & Statistics, DAC&FW website (http://eands.dacnet.nic.in). The information on Macro-Economic aggregates like GDP, personal disposable income, exchange rate and human population was collected from Reserve Bank of India website (http://dbie.rbi.org.in). The domestic and international price for groundnut was also taken from APEDA website and other published sources.

Analytical framework: To study change in direction of trade Markov Chain Analysis was employed and to study export supply function OLS regression followed by time series analysis was used to take care of spurious results obtained in OLS regression. For comparison whole study period was equally divided in two sub-period, period 1 (1996-97 to 2004-05) and period 2 (2005-06 to 2014-15).

Markov chain analysis: Major destinations were identified based on the average quantity exported to each country in respective periods. The Markov chain approach was employed to estimate transitional probability matrix (TPM). The element P_{ij} of this matrix indicates the probability that exports will switch from country i to country j with the passage of time. The diagonal P_{ij} measures the probability that the export share of a country will be retained. Therefore, diagonal element of the TPM pointed out the retention of an importing country to Indian groundnut export in respective

period. In Markov Chain analysis average export to a particular country is considered to be a random variable which depended only on its past exports to that country (For details refer Mahadevaiah *et al.,* 2005).

Export supply function analysis: The export supply function points out the influence of price and non-price factors on export of a commodity (Sato, 1977; Onkon and Sunday, 2014). The export of an agricultural commodity from a country mainly depends on its production, domestic demand, domestic and international prices along with socio-economic factors like GDP, exchange rate and personal disposable income. It can be written as:

$$\ln QEt = b_0 + b_1 \ln P_{t-1} + b_2 \ln DP_t + b_3 \ln EP_t + b_4 \ln ER_t + b_5 \ln GDP_t + b_6 \ln POP_t + b_7 \ln PDI_t + \mu_{it}$$

Where,

$\ln QE_t$= Groundnut export from India (in tonnes)

b_0= Intercept

$\ln P_{t-1}$= Previous year domestic production (in tonnes)

$\ln DP_t$= Domestic price (₹/qtl)

$\ln EP_t$= Export price (Dollar/tonnes)

$\ln ER_t$= Rupee exchange rate (₹/$)

$\ln GDP_t$= GDP in billion rupees at factor cost (base year : 2004-05)

$\ln POP_t$= Population in million

$\ln PDI_t$= Personal disposable income in rupees

b_1 to b_7= Regression coefficients

μ_{it}= Stochastic error term

Time series analysis: The recent developments in time series econometrics suggest that most time series are non-stationary. In case of non-stationary time series data, the common statistical tools are not appropriate and gives spurious results. In this study economic time series like GDP, exchange rate, domestic and export prices etc. trended over time. The regressions between trending series may produce significant results with high R2, but may be spurious or meaningless (Granger and Newbold, 1974). Therefore, it became necessary to check into the time series properties of the variables used in present study and to use the appropriate method of estimation based on the time series properties of the variables. Therefore, Engle and Granger (1987) two-step procedure was used in here. In first step, the order of integration between the data series was found out using ADF and Phillips-Peron stationarity test was followed by cointegration test using Johansen cointegration test. In the second step, vector error correction model (VECM) was employed to estimate cointegration equation and to specify the dynamic error correction mechanism or speed of adjustment to long run equilibrium.

RESULTS AND DISCUSSION

Scenario of groundnut trade: International trade in groundnut is very thin (less than 5-6% of global production) and concentrated due to high domestic consumption in major producing countries like India and China (Diop *et al.,* 2004). During study period 3.72 per cent of the world production was traded in international markets. From 1996-97 to 2014-15, global trade in groundnut increased at significantly higher rate (2.20%) than production (1.77%; Table 1). In above period, top five groundnut exporting countries in the world are China (22.66%), India (18.64%), Argentina (13.59%), USA (14.30%) and Netherland (5.99%), jointly accounted for more than three fourth of the world's total export. Export from India registered significantly high compound growth rate (CGR) of 18.33 and 11.25 per cent per annum during period 2 and overall period respectively. The volume of export from India has increased more than five times from 1.07 lakh tonnes in TE 1996-97 to 5.85 lakh tonnes in TE 2014-15. During 2005-06 to 2014-15, export increased at significantly higher rate making India the leading exporter to the world with 26.19 per cent share to world export. From figure 1, it can be seen that India's share to world export exhibited increasing trend since 2003-04 on regular basis with a maximum of 42 per cent share during 2011-12 made her top exporter to the world. During study period, the groundnut export from India increased by fourteen times in value terms. This confirmed the tremendous performance by Indian as of increased price groundnut from 2005-06 and onward. During period 2, the groundnut export from India increased in consistent way indicates optimistic future of Indian groundnut in world market in years to come (Table 2). Increase in value of Indian agricultural exports during 2010-11 was primarily because of higher exports of groundnut, sugar, molasses, cotton, guar gum meal, spices, maize, coffee, oil meal, castor oil, tea and jute etc (Sharma, 2013). During TE 2015-16, India exported 5.85 lakh tonnes groundnut, accounting at 3.96 lakh crore rupees to more than hundred countries, mainly shipped to Indonesia (33.32%), Vietnam (13.82%), Malaysia (11.41%), Philippines (10.10%) and Thailand (6.87%), jointly accounted for almost three fourth of total export from India in terms of quantity (Table 3).

Groundnut export from India exhibited significant growth and decreasing instability over the period indicates that India has emerged as reliable groundnut supplier to world market in study period (Table 2). Instability in groundnut export from India has decreased from 80 to 28 per cent in quantity and from 81 to 35 per cent in value from period 1 to period 2, respectively. Export value was more volatile than quantity

as it depends on so many others factors along with quantity exported. The decrease in instability in second period indicated that India became more regular exporter in period 2 than in period 1. Results indicated that during first five years after WTO the export from India showed decreasing trend followed by a revival subsequently as reported earlier by Sharma (2013) and Shah (2013). Similarly, during period 1, groundnut export to production share varied from 3.33 to 0.65 per cent. In period 2, India exported more than 6 per cent of her total production, with maximum of more than 11 per cent during 2011-12 and 12-13. As a result India became top exporter to world market in recent years with increased share in world's export (Table 3). Surge in export to production share in recent years depicts decreasing domestic consumption in India, because of shifting consumption pattern of edible oils in the country from traditional groundnut and rapeseed & mustard towards cheap imported palm and soya oils. From 2001-02 to 13-14, groundnut oil consumption has decreased from 12 to 2.13 per cent whereas palm oil consumption increased from 29 to 42 per cent (Meena *et al.,* 2015; Mehta, 2015).

Change in pattern of groundnut export from India: It is mostly traded as shelled (kernel), in-shell (pod) and prepared groundnut like, salted, blanched, preserved and peanut butter etc. During study period, 89 per cent in quantity and 92 per cent in value terms of the total groundnut export from the country was merchandised in shelled form (including both Hand Picked Selected (HPS) and Not Specified Elsewhere (NES), shelled groundnut). In-shell groundnut constituted 8 and 6 per cent share in quantity and value term respectively. Prepared groundnut contributed only 3 and 2 per cent in volume and value term, respectively during the study period. Peanut butter, a new product form of groundnut export basket since 2008-09 is gaining importance in recent years. Kernel export decelerated in period 1, but recorded tremendous recovery in period 2 with 21 and 35 per cent growth in quantity and value term respectively. Kernel export registered higher growth than in-shell export in overall period. Total groundnut export recorded higher growth in period 2 than period 1 and in overall (Table 4). Trading in kernel form is having higher export competitiveness than in-shell and prepared groundnut. It has freight advantage over in-shell groundnut as pod contains 30 per cent of shell, which has low economic value. Further kernels have more self-life compared to prepared peanut might be the reasons to export more in kernel form (Nautiyal, 2002; Meena, *et al.,* 2019). Instability in HPS kernels export came down over the period but instability in in-shell HPS increased confirms the increasing preference towards kernel export by the traders over the export of pod.

Table 1 Performance of groundnut export in term of quantity and share to world export in major exporting countries

Country	Particulars	Period 1 (1996-97 to 2004-05)			Period 2 (2005-06 to 2014-15)			Overall (1996-97 to 2014-15)		
		Mean	Growth	Instability	Mean	Growth	Instability	Mean	Growth	Instability
China	Export (Lakh tonnes)	3.58	10.14^{2}	0.39	2.23	-13.46^{2}	0.14	2.90	-4.59^{3}	0.28
	Production (Lakh tonnes)	129.3	5.00^{3}	0.09	150.1	3.40^{3}	0.06	139.7	2.34^{3}	0.08
	% Share to world export	29.25	10.35^{2}	0.38	16.06	-17.90^{3}	0.13	22.66	-6.64^{2}	0.28
India	Export (Lakh tonnes)	1.35	-1.95^{NS}	0.89	4.05	18.33^{3}	0.25	2.70	11.52^{3}	0.58
	Production (Lakh tonnes)	69.8	-3.20^{NS}	0.39	72.4	1.32^{3}	0.40	71.1	0.08^{3}	0.38
	% Share to world export	11.08	-1.76^{NS}	0.88	26.19	12.25^{3}	0.20	18.64	9.12^{3}	0.56
Argentina	Export (Lakh tonnes)	1.73	-12.16^{3}	0.30	1.90	5.89^{2}	0.26	1.81	0.35^{NS}	0.30
	Production (Lakh tonnes)	3.78	-5.66^{NS}	0.48	6.27	9.92^{3}	0.26	5.03	4.82^{3}	0.38
	% Share to world export	14.30	-12.00^{2}	0.33	12.88	0.45^{NS}	0.26	13.59	-1.81^{NS}	0.30
USA	Export (Lakh tonnes)	1.84	-3.88^{NS}	0.38	2.00	6.24^{NS}	0.31	1.92	0.78^{NS}	0.34
	Production (Lakh tonnes)	17.3	1.45^{NS}	0.17	20.0	1.87^{3}	0.35	18.6	1.49^{3}	0.27
	% Share to world export	15.04	-3.70^{NS}	0.33	13.56	0.79^{NS}	0.36	14.30	-1.38^{NS}	0.34
Netherlands	Export (Lakh tonnes)	0.63	-4.96^{2}	0.19	1.02	9.65^{3}	0.12	0.83	4.47^{3}	0.16
	% Share to world export	5.22	-4.78^{3}	0.19	6.76	4.03^{NS}	0.09	5.99	2.22^{2}	0.14
Others	Export (Lakh tonnes)	3.25	-2.42^{3}	0.24	3.61	4.09^{NS}	0.17	3.43	1.12^{3}	0.20
	Production (Lakh tonnes)	79.5	2.88^{3}	0.04	101.4	3.47^{3}	0.04	90.4	2.84^{3}	0.04
	% Share to world export	26.63	-2.23^{3}	0.20	24.55	-1.25^{NS}	0.17	25.59	-1.05^{NS}	0.18
World	Export (Lakh tonnes)	12.18	-0.19^{NS}	0.08	14.80	5.41^{3}	0.09	13.49	2.20^{3}	0.09
	Production (Lakh tonnes)	336.4	2.26^{3}	0.08	388.6	2.22^{2}	0.09	362.5	1.77^{3}	0.08

Note: 3, 2, 1 and NS indicate significant at less than 1,5,10 per cent level of significance and non-significant respectively.
Source: Calculations based on data from FAO (2016).

Table 2 Export performance of Indian groundnut in quantity ('000 Tonnes) and value (in ₹ Crore) term

Particulars		Production	Export	Value of produce	Export Value
	Mean	6849.92	142.65	12843.03	360.68
Period 1	Growth	-2.35 NS	-0.66 NS	-4.09 NS	3.35 NS
	Instability	0.41	0.80	0.42	0.81
	Mean	7167.39	436.93	14023.29	2438.44
Period 2	Growth	0.69 NS	15.34 3	4.03 NS	28.26 3
	Instability	0.44	0.28	0.34	0.35
	Mean	7017.01	297.53	13464.22	1454.24
Overall	Growth	0.17 NS	11.31 3	0.83 NS	19.44 NS
	Instability	0.42	0.56	0.37	0.58

Source: Calculations based on data from Directorate of Economics & Statistics, DAC & FW (2016) and MOSPI (2016).

Table 3 Groundnut export from India and its share to production and world export

Year	Production (000 tonnes)	Export (000 tonnes)	Export value (₹ Crore)	Export to production share (%)	India to world export Share (%)
1996-97	7589.41	151.35	331.59	1.99	11.43
1997-98	7370.40	245.13	566.28	3.33	19.87
1998-99	8980.00	58.26	139.62	0.65	5.03
1999-00	5250.00	158.11	371.78	3.01	13.41
2000-01	6410.00	137.07	316.41	2.14	9.97
2001-02	7027.50	112.81	250.93	1.61	8.12
2002-03	4121.10	67.85	178.19	1.65	4.75
2003-04	8126.50	176.11	544.27	2.17	13.58
2004-05	6774.40	177.15	547.01	2.62	13.59
2005-06	7993.30	190.05	513.7	2.38	13.38
2006-07	4863.50	251.43	798.46	5.17	19.10
2007-08	9182.50	269.59	1054.08	2.94	19.75
2008-09	7168.10	297.89	1239.02	4.16	22.78
2009-10	5428.49	340.25	1425.93	6.27	24.14
2010-11	8265.78	433.75	2178.38	5.25	27.42
2011-12	6963.70	832.62	5246.45	11.96	42.02
2012-13	4693.88	535.64	4065.38	11.41	36.88
2013-14	9713.90	509.66	3187.69	5.25	30.27
2014-15	7401.71	708.39	4675.35	9.57	NA

Source: Data compiled from DAC & FW, DGCIS and FAO.

Table 4 Performances of groundnut export in different forms in quantity (000 tonnes) and value (₹ Crore) term

Forms (HS code)	Particulars	Average			Growth (%)			Instability index		
		Period 1	Period 2	Overall	Period 1	Period 2	Overall	Period 1	Period 2	Overall
Shelled HPS (12022001)	Quantity	99.91	402.04	258.93	-8.46	20.99	13.36	0.80	0.30	0.59
	Value	253.99	2296.58	1329.03	-3.56	34.62	21.49	0.81	0.36	0.61
Shelled N.E.S. (12022009)	Quantity	16.89	10.15	13.34	4.59	-10.75	-6.88	1.04	0.83	0.91
	Value	42.43	42.66	42.55	7.35	-0.53	-0.07	1.09	0.84	0.94
Shelled total (HPS+ N.E.S.)	Quantity	116.80	412.19	272.27	-6.16	18.96	12.38	0.83	0.29	0.59
	Value	296.41	2339.24	1371.58	-1.80	32.39	20.50	0.84	0.36	0.62
In-shell HPS (12021001)	Quantity	21.35	14.21	17.59	33.15	-28.47	-6.65	0.54	1.04	0.90
	Value	52.98	54.10	53.57	37.80	-22.61	0.16	0.51	0.99	0.85
In-shell N.E.S. (12021009)	Quantity	4.50	10.53	7.67	32.44	-2.73	5.56	1.07	1.73	1.42
	Value	11.29	45.11	29.09	35.58	5.67	12.42	1.17	1.77	1.48
In-shell Total (HPS + N.E.S.)	Quantity	25.85	24.74	25.26	33.52	-9.67	1.26	0.52	0.76	0.67
	Value	64.27	99.21	82.66	38.28	-3.37	8.29	0.51	0.44	0.50
Prepared/Preserved (20081100)	Quantity	11.21	8.74	9.91	-7.42	-2.95	-0.65	1.08	0.80	0.92
	Value	26.41	47.03	37.26	-4.95	11.72	8.14	1.03	0.76	0.87
Gross	Quantity	142.65	436.93	297.53	-0.66	15.34	11.31	0.80	0.28	0.56
	Value	360.68	2438.44	1454.24	3.35	28.26	19.44	0.81	0.35	0.58

Source: Computed from DGCIS, Ministry of Commerce and Industry, Govt. of India data.

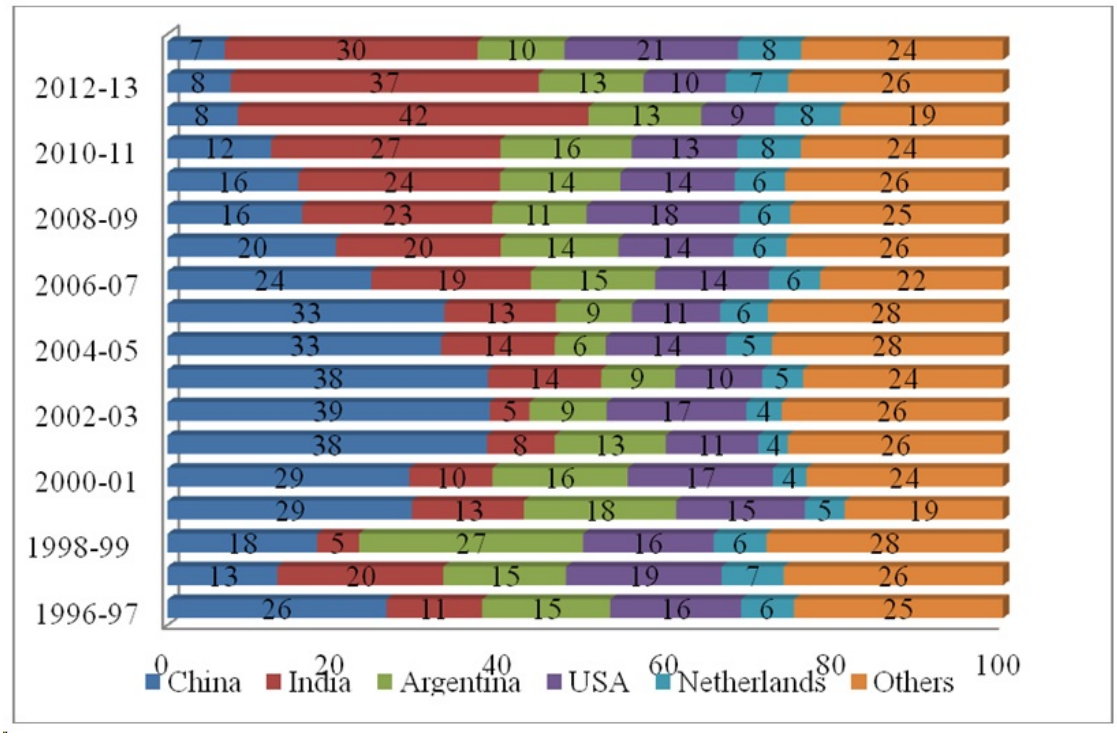

Fig. 1. Proportionate shares (%) of major exporting countries to world groundnut export (in quantity term)

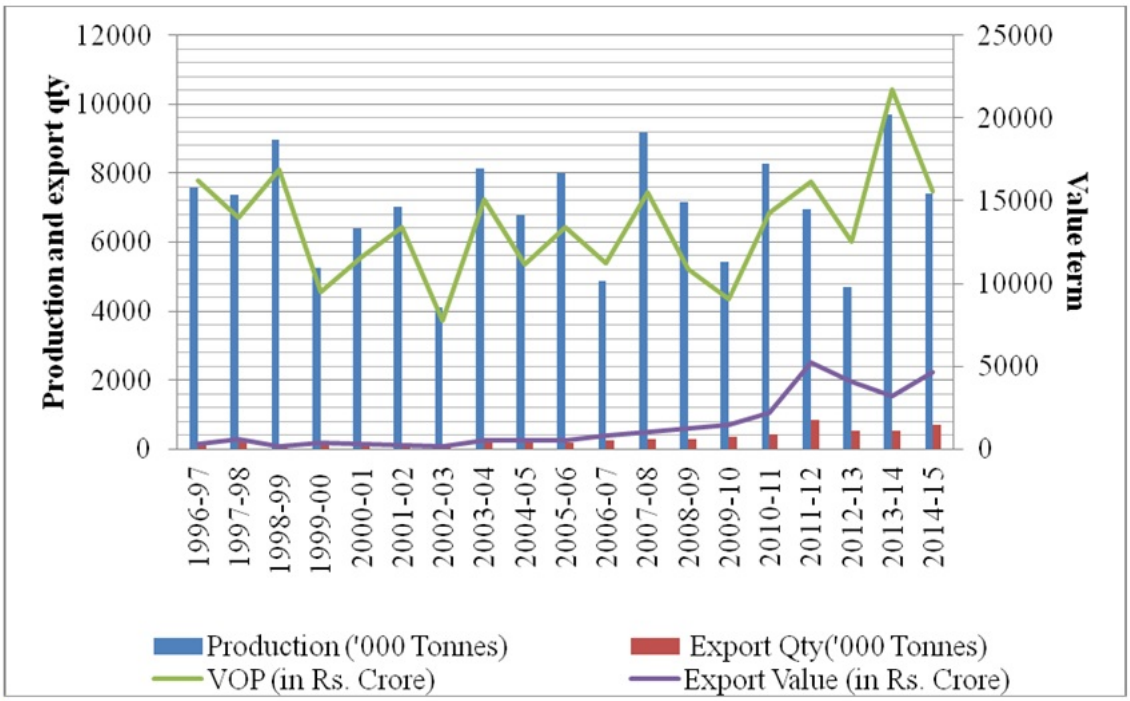

Fig. 2. Production and export of groundnut from India in quantity (primary axis) and value term (secondary axis)

Table 5 Change in Indian groundnut export destinations during 1995-96 to 2014-15

Country	1995-96 to 2004-05				Country	2005-06 to 2014-15			
	Particulars	Average	Growth	Instability		Particulars	Average	Growth	Instability
Indonesia	Quantity	61.39	0.57	0.83	Indonesia	Quantity	164.98	8.37	0.28
	% Share	44.79	0.53	0.37		% Share	41.23	-6.04	0.27
Malaysia	Quantity	19.29	21.29	0.52	Malaysia	Quantity	56.39	8.57	0.19
	% Share	14.34	21.25	0.36		% Share	14.18	-5.87	0.30
Philippines	Quantity	8.26	-0.33	1.29	Philippines	Quantity	47.22	12.99	0.36
	% Share	5.56	-0.37	0.75		% Share	11.58	-2.03	0.43
Singapore	Quantity	5.20	-7.65	0.82	Singapore	Quantity	6.03	-1.10	0.13
	% Share	3.77	-7.68	0.34		% Share	1.72	-14.25	0.34
UK	Quantity	13.76	-10.94	1.14	Vietnam	Quantity	61.10	136.49	1.89
	% Share	9.02	-10.98	0.49		% Share	9.06	105.04	1.69
Netherland	Quantity	3.84	-12.77	1.92	Thailand	Quantity	14.47	211.20	4.99
	% Share	2.08	-12.80	1.36		% Share	2.67	169.81	5.03
Egypt	Quantity	2.38	-25.74	1.55	Pakistan	Quantity	12.04	37.14	0.94
	% Share	1.96	-25.25	1.68		% Share	2.51	18.90	0.97
Sri Lanka	Quantity	2.37	9.28	0.69	China	Quantity	11.03	174.78	3.35
	% Share	2.15	9.23	1.13		% Share	1.90	138.24	3.23
USA	Quantity	2.36	-26.35	8.89	Ukraine	Quantity	8.95	17.22	0.61
	% Share	1.41	-16.35	5.64		% Share	2.10	1.63	0.61
Russia	Quantity	2.35	-59.26	8.20	UAE	Quantity	7.28	4.68	0.30
	% Share	1.53	-50.39	4.62		% Share	1.91	-9.24	0.46
Others	Quantity	19.30	-4.10	0.76	Others	Quantity	47.43	11.95	0.56
	% Share	13.39	-4.13	0.67		% Share	11.14	-2.94	0.42

Note: Qty. indicates quantity in '000 tonnes and share indicate per cent share to total groundnut export from India.
Source: Computed from DGCIS, Ministry of Commerce and Industry, Govt. of India data

Table 6 Transitional probability matrix of Indian groundnut export during 1995-96 to 2014-15

Country	Indonesia	Malaysia	Philippines	Vietnam	UK	Thailand	Ukraine	Singapore	Pakistan	UAE	Others
Indonesia	52.94	11.76	0.00	11.76	0.00	0.00	0.00	0.00	5.88	0.00	17.65
Malaysia	11.11	22.22	22.22	0.00	5.56	0.00	0.00	0.00	11.11	0.00	27.78
Philippines	11.11	11.11	16.67	0.00	11.11	5.56	5.56	0.00	0.00	16.67	22.22
Vietnam	11.11	0.00	0.00	11.11	5.56	27.78	0.00	5.56	27.78	11.11	0.00
UK	0.00	16.67	22.22	0.00	5.56	5.56	22.22	11.11	0.00	11.11	5.56
Thailand	0.00	0.00	0.00	11.11	22.22	22.22	11.11	0.00	16.67	5.56	11.11
Ukraine	0.00	0.00	16.67	11.11	11.11	11.11	11.11	11.11	5.56	22.22	0.00
Singapore	0.00	0.00	11.11	5.56	5.56	5.56	5.56	44.44	5.56	16.67	0.00
Pakistan	0.00	6.00	0.00	33.33	11.11	11.11	22.22	0.00	16.67	0.00	0.00
UAE	0.00	11.11	0.00	5.56	11.11	5.56	22.22	27.78	5.56	11.11	0.00
Others	16.67	22.22	11.11	5.56	11.11	5.56	0.00	0.00	5.56	5.56	16.67

Source: Computed from DGCIS, Ministry of Commerce and Industry, Govt. of India data

Change in direction of groundnut export from India: Direction and destinations of export are crucial to define India's export potential. A sluggish growth experienced by export partners indicates that demand for Indian exports is likely to be constrained in near future. Therefore, to find out how the share of traditional export partners has altered and how the country has succeeded in capturing new markets for its export in post WTO period was assessed. Table 5 shows that 87 and 89 per cent of the total quantity exported during period 1 and 2, respectively was concentrated in top ten importing countries *viz.,* Indonesia (43.95%), Malaysia (15.08%), Philippines (8.85%), Vietnam (3.36%), United Kingdom (UK; 5.28%), Thailand (1.40%), Ukraine (2%), Singapore (2.46%), Pakistan (1.15%) and United Arab Emirates (UAE; 1.65%) Exports to Indonesia, Malaysia, Philippines and Singapore jointly constituted 68.46 and

68.71 per cent share to total export in period 1 and 2 respectively. India lost its share in the UK, Netherland, Egypt, Sri Lanka, the USA and Russia markets in period 2, but exported to new partners like Vietnam, Thailand, Pakistan, China, Ukraine and UAE during same period. The highest growth in export during period 1 was observed in export to Malaysia whereas highest negative growth was found in export to Russia in this period. Highest instability index was observed in export to USA (8.89) followed by Russia (8.2) due to tightening of the safety measures with the clients. In period 2, Thailand, Pakistan, China, Ukraine, UAE and Singapore joined as premier customer to India. China, the largest producer of groundnut in world, also imported Indian groundnut to meet its domestic demand in period 2. It was quite noticeable that newly joined partners to India showed very high degree of instability varying from 500 per cent in Thailand, 300 per cent in case of China to 200 per cent in Vietnam. In case of old regular trading partners like, Indonesia (28%), Malaysia (19%), Philippines (36%), and Singapore (13%), instability was very low compared to new partners.

Similar to the earlier findings (Mahadevaiah *et al.,* 2005; Savadatti, 2006; Dominic, 2008; Sharma, 2013; Varghese, 2014, Adhikari *et al.,* 2016) present study also revealed narrowing market for Indian groundnut in terms of distance. The sphere of Indian groundnut export has shifted from European to Asian countries from period 1 to period 2. In period 1 Indian groundnut was landing into the United Kingdom, Egypt, Netherland and USA along with major markets like Indonesia and Malaysia, whereas in second period these long distance markets were replaced by near markets namely, Vietnam, Thailand, China, UAE and Pakistan which clearly indicated the narrowing trade sphere from far distant to nearby markets, with increasing export in quantity term. It was mainly because of strict sanitary and phytosanitory measure adopted by European countries and another is higher freight charges to these far destinations like United Kingdom, Netherland, Egypt (Jagdambe, 2016). Low retention share of USA and UK might be the result of change in trade policies in these countries in post WTO period. Alfatoxin level which was a major setback to groundnut exports from India to the European Union. To manage aflatoxin contamination at different stages in groundnut supply chain it is immensely needed to give more emphasis for good agricultural practices in groundnut production right from farmers till end consumer through proper handling of produce by industry people. High moisture content in export consignment is the root cause for aflatoxin problem (ICRISAT, 2016; Mukherjee *et al.* 2019). European market is the largest importer of groundnut in world but India is having very low or negligible share in their markets. It calls for India to target these markets by meeting their quality standards. It will strengthen India's groundnut competitiveness in world market.

Markov Chain Analysis was done to examine structural change in groundnut export from India. Top ten importing countries were taken to analyse the shift in direction while export to remaining countries were pooled under others category. TPM of groundnut export during period1, 2 and overall was estimated but due to space limitations TPM for overall period is only presented here in Tables 6. It was evident from TPM for period 1 that Indonesia was the most loyal importer of Indian groundnut as reflected through high probability of retention of 78 per cent followed by Egypt and Sri Lanka. Malaysia was not a stable importer in period 1, even though quantity imported by Malaysia was high about 20 thousand tonnes in period 1. Similar case was observed in case of Indian turmeric export to USA. USA was the second top destinations for India turmeric between 1990 and 2007 but it retained zero per cent share of its previous export (Angles *et al.,* 2011). During period 2, Singapore was identified as biggest loyal market for Indian groundnut. It retained 80 per cent share of lagged years. Indonesia continued to be a premier customer with 67 per cent retention over previous year in period 2. Vietnam and Malaysia were next loyal in this period, retained 30 and 50 per cent export share over previous year respectively. Similarly, Varghese in 2014 also identified Indonesia and Malaysia as most loyal markets for groundnut kernel HPS with high retention probabilities of 59 and 43 per cents respectively from 2001 to 2008. TPM for overall period i.e. from 1995 to 2015 found Indonesia as most loyal destination because it retained 52.94 per cent of its previous year export share. Singapore emerged as second steady market. Malaysia and Thailand also retained more than one fifth of their previous year export. Others countries retention improved from 20 per cent in period 1 to 30 per cent in period 2 indicating that non-traditional small importer retained their share in addition to gain from larger importer like Malaysia and Philippines (Table 6). Retention share of other countries improved in period second compared to period one which indicated the increasing preference of Indian groundnut in new importing countries.

Factors influencing groundnut export from India: Agricultural export from a country is subjective to various price and non-price factors (Haleem *et al.* 2005; Mythili, 2007; Ranjan and Rai 2007; Kumawat and Prasad 2012; Kannan 2013; Okon and Sunday 2014; Saxena *et al.* 2015). It is mainly influenced by domestic demand in exporting country and the relative prices she receives for exports. The export supply function indicates the relative influence of relevant price and non-price factors and associated policies in stimulating the supply of exports (Islam and Subramanian, 1989). There is a negative link between domestic demand and exports from a country. Changes in domestic prices in relation to export prices influence an exporter's decision to

supply goods equally. The interaction between prices and the exchange rate plays a crucial role in determining India's exports because any change in exchange rate affects both the prices and the competitiveness of exports (Shah, 2013). Theoretically, a depreciating currency stimulates exports by making it cheaper abroad providing a quick boost in competitiveness.

The export function of Indian groundnut was estimated to identify the factors that determine the groundnut export from India. The results (Table 7) indicated that the factors included in the models explain72 per cent variation in export volume. GDP at factor cost (04-05 base year) and population had a significant positive influence on the volume of groundnut exports, whereas exchange rate had a significant negative influence on the volume of export. Rest of the variables were found insignificant. The estimation suggested that with 1per cent increase in the GDP, exports increased by 0.96 percent. There was inverse relationship between exchange rate and export and proportional relationship between population and export. Theoretically, a depreciating currency stimulates exports while with increase in population the available surplus should decrease resulting in the decrease in export. Therefore, the regression results seem to be spurious as they were found against established hypotheses. Hence, it was analysed further considering the time series properties of the variable used in model.

Regressions between trended series may produce significant results with high R^2, but may be spurious or meaningless (Granger and Newbold, 1974). So it became appropriate to look into the time series properties of the variables used in the estimation and to use estimation method based on the time series properties of the variables. Hence, appropriate time series techniques namely, ADF and Phillips Peron stationarity test, Johansen Cointegration test and Vector Error Correction Model were used.

Non-stationarity causes a serious inference problem therefore stationary test was employed for export, production, domestic and export prices, GDP, population and personnel income before co-integration tests. To examine the univariate time series properties of the variables and to confirm that all the series are non-stationary and integrated of the same order, ADF and Phillips Peron unit root test were used. Both the tests produced almost similar results (Table 8). Results showed that export, domestic price, export price, exchange rate and population series were non-stationary at the level, as the unit root test statistic was less than the critical value at 5% level. At first difference, the null hypothesis of unit root could be rejected for all the variables i.e. they become stationary at the first difference. But lagged year production was found stationary at level itself whereas GDP and personal disposable income did not become stationary even after second differencing. When the unit root null hypothesis ($\beta=1$) was rejected against the alternative hypothesis ($\beta<1$), it meant that the non-stationary variables Y_t and Z_t co-integrated over long run. Therefore, both the unit root test namely ADF and Phillips Peron results confirmed that export, domestic price, export price, exchange rate and population variables were integrated of the same order i.e. [I(1)]. It confirmed the precondition for cointegration i.e. non-stationarity at level and integrated of same level. It confirmed a long-run or equilibrium relationship between groundnut export, its domestic price, its export price, exchange rate and population. After establishing non-stationarity of variables at level and were integrated of same order, Johansen co-integration test was employed to check long-run relationship among these integrated variables (Johansen, 1988). Johansen cointegration test as sequential test procedure was employed to determine the number of cointegrating relations among the variables. Each row of the Tables 9 and 10 tested a different null hypothesis. The results based on Trace statistic test and Eigen value statistics, and Johansen cointegration test indicated 5 cointegrating equations/vectors at 5 per cent level of significance, and it suggested that there was high long run association between export, domestic price, export price, exchange rate and population. The Johansen test was proceeded to estimate VECM to measure the magnitude of speed of adjustment to the long-run equilibrium. VECM produced theoretically correct results against OLS estimates. The cointegration equation (Table 11) showed that export of groundnut from India increased significantly with export price and exchange rate. On the other hand export decreased significantly with increase in domestic price and increase in population (due to increased domestic consumption). Similar results were also obtained by Sengupta and Roy (2011) in case of chilli & pepper, banana and walnut export which decreased with domestic prices. In case of rice export, Adhikari *et al.* (2016) found that rice export from India increased with exchange rate and international price and decreased with domestic prices and population . The coefficient of the speed of adjustment for groundnut export from India was negative and statistically significant. It reflects the adjustment to the long-run equilibrium from short run shocks, implied that groundnut export tends to converge to equilibrium in long-run after short run shocks. Hence, it could be concluded that domestic and international prices, exchange rate and population played a significant role in groundnut export from India during 1995 to 2015.

This study indicated that groundnut export from India increased significantly during 1995 to 2015. India has emerged as top, reliable and consistent supplier of groundnut to world market. The export market sphere has narrowed from European to Asian countries amid tightening of SPS measures and increasing cut-throat competition in world market. There is needs to adopt good and efficient production and manufacturing practices by all the

stakeholders in peanut supply chain. Export from India significantly depends on domestic and export prices, exchange rate and population. Therefore, export promotion policies should be articulated to increase groundnut export from India accordingly.

Table 7 OLS estimates of groundnut export supply function; Dependent variable-Ln export quantity

Particulars	Dependent variable- Ln Export volume (Mt)	
	Coefficients	t Stat
Intercept	0.21	0.03
Ln Production (t-1; Mt)	0.12	0.25
Ln Domestic price (₹/qtl)	0.16	0.29
Ln Export price ($/tonnes)	0.13	0.29
Ln Exchange rate (₹/$)	-2.25**	-2.25
Ln GDP at factor cost (₹ Billion; base year 04-05)	0.96*	2.08
Ln Population (in million)	1.04**	2.30
Ln Personal disposable income (₹)	-0.07	-0.31
Adjusted R square	0.72	

Note: *,**, *** Indicates that the variable is significant at less than 10%, 5% and 1% level respectively

Table 8 Results of ADF and Phillips-Peron Unit Root test

Series	ADF Fisher		Phillips-Peron Fisher	
	Level	First difference	Level	First difference
Export	0.78	0.00	0.85	0.00
Production (t-1)	0.00	0.00	0.00	0.00
Domestic price(₹/qtl)	0.77	0.05	0.77	0.05
Export price($/tonnes)	0.94	0.00	0.53	0.03
Exchange rate(₹/$)	0.92	0.04	0.92	0.04
GDP (₹ Billion; base year 04-05)	1.00	1.00	1.00	1.00
Population(in million)	0.96	0.00	0.97	0.00
Personal disposable income (₹)	1.00	1.00	1.00	0.14

Table 9 Result of Johansen Cointegration test using Trace statistics

Unrestricted Cointegration Rank Test (Trace)				
Hypothesized No. of CE(s)	Eigenvalue	Trace Statistic	0.05 Critical Value	Prob.**
None *	0.95	125.58	69.82	0.00
At most 1 *	0.85	75.88	47.86	0.00
At most 2 *	0.74	43.90	29.80	0.00
At most 3 *	0.54	20.74	15.49	0.01
At most 4 *	0.35	7.42	3.84	0.01
Trace test indicates 5 cointegratingeqn(s) at the 0.05 level				

Table 10 Result of Johansen Cointegration test using Eigen value statistics

Unrestricted Cointegration Rank Test (Maximum Eigenvalue)				
Hypothesized No. of CE(s)	Eigenvalue	Max-Eigen Statistic	0.05 Critical Value	Prob.**
None *	0.95	49.70	33.88	0.00
At most 1 *	0.85	31.98	27.58	0.01
At most 2 *	0.74	23.16	21.13	0.03
At most 3 *	0.54	14.31	14.26	0.07
At most 4 *	0.35	7.42	3.84	0.01
Max-eigenvalue test indicates 5 cointegratingeqn(s) at the 0.05 level				

Table 11 Results of Vector Error Correction Model

CointegratingEq:	CointEq1		
Export(-1)	1.000000	Standard errors	t-statistics
Domestic price(-1)	-219.62***	-17.05	-12.88
Export price(-1)	54.74*	-39.97	1.37
Exchange rate(-1)	7792.44**	-2535.74	3.07
Population(-1)	-2537.44***	-88.83	-28.56
C	270651.8		
Error Correction:	D(Export)		
Speed of adjustment	-1.253834**	(0.66727)	[-1.87904]
R-squared	0.731588		
Adj. R-squared	0.496727		
F-statistic	3.114984		
Log likelihood	-201.4536		
Akaike AIC	26.18170		
Schwarz SC	26.56800		

Note: *,**, *** Indicates variable is significant at the 10, 5 and 1% level respectively.

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Effects of process parameters on recovery and quality of groundnut oil

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ABSTRACT

In the present study effects of particle size and heat treatment on recovery and quality of groundnut oil was determined. Various physico-chemical parameters *viz.,* colour, density, iodine value, peroxide value and saponification value were determined using standard methods. The results revealed that size reduction of groundnut kernels increased the oil yield, but it deteriorated the quality of oil, whereas heat treatment (60°C for 1 h), improved the oil yield as well as quality. The oil yield was highest (48.14%) for finely ground kernels with heat treatment and was lowest for whole kernels without heat treatment (35.20%). The saponification value, iodine and peroxide value of oil extracted from the whole kernels with heat treatment was found to be the minimum 106.34 mg KOH/g, 99.24 mg I_2/g oil and 0.65 M eq/kg respectively. The maximum value of oleic acid content and linoleic acid content of 51.39% and 31.44% was found in the oil sample of raw groundnut kernels with heat treatment respectively. It was observed that the groundnut oil extracted from whole kernels with heat treatment (60°C for 1 h) is superior in quality in terms of colour, fatty acid composition with a comparatively longer shelf life and resistance to rancidity and oxidation.

Keywords: Fatty acid profile, Groundnut oil, Oil expression, Physico-chemical properties

Groundnut (*Arachis hypogaea* L.) is one of the most important legume-oilseed crops of the world. India stands first in terms of acreage (4.94 m ha) and second in terms of production (6.69 MT) of groundnut in the world (FAOSTAT, 2018). The productivity levels of groundnut in India remain lower than the world average mainly because of its cultivation as a rainfed crop on >80% acreage (Rathankumar *et al.,* 2010). It is grown mainly in Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu and Uttar Pradesh. In Punjab, it was cultivated only on one thousand hectares in a few pockets of the state. Groundnut is a rich source of nutrients such as oil, protein, carbohydrates and minerals and therefore, is used in various forms. Groundnut kernel is also rich in calories (Patil *et al.,* 2014) and is highly digestible with high biological value. Groundnut is particularly valued for its protein content (26%) and contains 'lysine' which is lacking in cereals. Globally 50% of the groundnut produced is used for oil extraction and about 40% is used directly as food, raw or processed as snack (Birthal *et al.,* 2010). Groundnut kernel (seed) contains 45% to 55% oil (Perez and Arguello, 1995) which is rich in mono-unsaturated (oleic acid, 48%) and polyunsaturated (linoleic acid, 32%) fatty acids. Groundnut oil is a kind of light yellow transparent edible oil with clear colour, pleasant fragrance, good taste and easy to digest. Groundnut oil is extensively used as a cooking medium both as refined oil and vanaspati ghee in addition to its use in manufacturing cosmetics, soap making and lubricants, olein stearin.

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Generally, the oil from oilseeds can be extracted by different methods *viz.,* pressing, solvent extraction, aqueous extraction, enzymatic, ultrasound assisted extraction methods (Shende and Sidhu, 2014; Shende and Sidhu, 2016; Gayas *et al.,* 2016; Datt and Kaur, 2019). Oil pressing which refers to the use of mechanical forces to squeeze oil out of kernels of groundnut, is the most frequently applied method of oil extraction (Khan and Hannah, 1984). Hot pressing is the traditional process of extraction of groundnut oil in which the kernels are steamed, fried and pressed, usually at a temperature above 120°C to give high rate of oil output. But because of the high production temperature, the groundnut protein is severely denatured resulting in loss of high amount of nutrients. Cold pressing method (temperature below 60°C) involves removal of skin (pericarp) before pressing and is beneficial as it preserves nutrients and groundnut protein in the oil.

Continuous mechanical expression process is the most commonly encountered process in the processing industry. However the comparison between industrial and laboratory performances is still lacking. Mechanical extraction is the most widely used method in literature as it is the cheapest method in the industry and is the healthiest way to remove oil without polluting the grain sample. With respect to oil yield, screw press has an advantage over hydraulic press for churning out slightly higher yields of oil because of their continuous mode of operation (Arisanu, 2013). Mechanical press (manual or powered) meant for small (laboratory) scale oil extraction is simple, safer and involves fewer steps compared to the other methods of extraction of oil (Oyinlola *et al.,* 2004). On the industrial scale, industrial machines or expellers are used for the purpose of extracting vegetable oil

mechanically. The yield of oil depends on the number of passes in the expeller and the amount of pressure applied on the kernels. However, there is no scientific information on the effects of particle size and heat treatment on the recovery of oil and its quality. Keeping all these facts in view, the present study was undertaken to study the effect of pre-treatments on the recovery and quality of groundnut oil.

MATERIALS AND METHODS

Sample preparation: The most popular variety of groundnut grown in the Punjab state 'SG 99' was procured from Department of Plant Breeding, Punjab Agricultural University, Ludhiana. About 40 kg of groundnut was decorticated using manual groundnut decorticator. Clean and healthy kernels were selected for the study. The size reduction of the sample was done using a hammer mill after adjustment of the aperture of the mill. The size distribution of particles was done using sieve shaker (Macro Scientific Works Pvt. Ltd.) equipped with standard Tyler sieves series with opening sizes 5.6 mm, 2.8 mm, 1.4 mm, 710 µ, 355 µ and 180 µ followed by a collection pan. The sample was shaken for 10 min and the mass retained on each sieve and in the pan was weighed. The material retained on the top three sieves was considered as coarse particles (1.4 to 5.6 mm), and the material retained on the next three sieves and on the pan (1.4 to 0.180 mm) was considered as fine particles in the present study.

Pre-treatment of sample: The sample after grinding and sieve analysis i.e. whole kernels, coarse and fine groundnut kernels were dried in a tray dryer at 60°C for one hour (Makeri *et al*., 2011). The initial moisture content and final moisture content after drying of the samples was measured as 10±0.65% and 7±0.5% respectively. The samples were packed in an air tight container and stored in ambient conditions for further experimentation. The experimental scheme for extraction of oil from groundnut kernels is depicted in Table 1.

Table 1 Different treatments for extraction of oil from groundnut kernels

Treatment	Description
T1	Coarse kernels with heat treatment
T2	Coarse kernels without heat treatment
T3	Fine kernels with heat treatment
T4	Fine kernels without heat treatment
T5	Whole kernels with heat treatment
T6	Control sample (whole kernels without treatment)

Extraction of oil from groundnut kernels: Oil was extracted from the pre-treated samples of groundnut kernels using the oil expeller (Crompton Greaves Limited, India). The sample fed through the hopper was crushed and transported by a rotating screw in a press barrel. Continuous transport of material by the screw shaft caused pressure to increase to the needed level, which increased friction inside the screw press and generated heat which in turn lowered viscosity of the oil in the crushed seeds increasing the oil flow rate. The total oil was extracted after passing the material in the expeller for three times. The oil and cake were collected at the oil outlet and press cake exit /choke gap, respectively. The time taken for oil extraction and oil yield was determined.

$$\text{Oil yield (\%)} = \frac{[\text{Weight of oil (g)} \times 100]}{\text{Weight of sample (g)}}$$

Physico-chemical characteristics of groundnut oil: Different physico-chemical characteristics *viz*., saponification value, iodine value, peroxide value and colour of groundnut oil extracted by varying different process parameters was determined as under:

Iodine value: Iodine value was determined by method suggested by AOAC, 2000. About 2g of oil sample was taken in a dry glass stopper bottle of 250 ml capacity and 10 ml of carbon tetrachloride was added to the bottle containing oil. Fifteen ml of potassium iodide (10%) and 100 ml of water was added and then titrated with 0.1M sodium thiosulphate solution using starch as indicator just before the end point. Iodine value was calculated from the formula:

$$\text{Iodine value (\%)} = \frac{[(V2-V1) \times 1.269]}{\text{Weight of sample (g)}}$$

Where, V2 = titre value for blank
V1 = titre value for sample

Peroxide value: Peroxide value was evaluated as per the method suggested by AOAC 2000. About 2g of oil sample was taken in a tube to which one gram of powdered potassium iodide with 20 ml of solvent mixture (acetic acid and chloroform) was added. The tube containing the sample was then placed in boiling water for 30 seconds. The content was poured into a flask containing 20ml of 5% iodide solution. The tube was then washed and titrated with 0.002 N sodium thiosulphate solution using starch as an indicator. A blank was prepared alongside the oil samples. Peroxide value was obtained using the formula:

$$\text{Peroxide value (Meq/kg)} = \frac{[2 \times (V1-V2)]}{\text{Weight of sample (g)}}$$

Where, V2 is the value of blank titre and V1 is the value of sample titre value

Saponification value: The saponification value was determined according to the titrimetric method of AOAC 2000. Two gram of oil sample was taken in a conical flask to which 25ml of alcoholic potassium hydroxide was added. Solution was heated in water for one hour. One ml of phenothphalein was added and titrated with 0.5NHCL. The value was calculated by the formula:

$$\text{Saponification value} = \frac{[56.1 \times (A-B)]}{W}$$

Where,
N = normality of HCL acid used
A = Volume of H_2SO_4 used in blank titration
B = Volume of H_2SO_4 used in titration of the sample
56.1 = Equivalent weight of potassium hydroxide
W = Weight of oil used

Colour value: Colour properties of groundnut oil were determined by Lab Colorimeter. Colour was measured in terms of 'L*', 'a*' and 'b*'. Sample was completely filled in the petri dishes so that no light was allowed to pass during the measuring process. Value 'L' represents lightness, 'a' represents redness or greenness while 'b' represents blueness or yellowness. The values are presented in Table 3. Colour is a sensory attribute of food that can be used to determine its quality and consumer acceptability. The colour values can be recorded using a colorimeter which gives L*, a* and b* values. L* value indicates degree of lightness from black to white (range = 0 - 100). Negative b* is blue, positive b* is yellow; negative a* is green and positive a* is red. Both a* and b* values range from -128 to +127. These values can be used to calculate hue and chroma (also called saturation).

$$\text{Hue} = \tan^{-1}(b/a)$$
$$\text{Chroma} = (a^2+b^2)^{0.5}$$

Hue usually refers to as colour. So red, green, blue etc. are actually hue rather than simply color. Color is more complex, comprising of not only hue but also variations in lightness and intensity. Chroma refers to how intense the hue is.

Density of oil: Bulk density was determined by taking a known weight (100 g) of sample in a measuring cylinder and measuring its volume (Mohsenin, 1980):

$$BD = \frac{w}{v}$$

Where, BD= bulk density (g/ml), w= weight of sample (g), v= volume of sample (ml)

Determination of fatty acid composition of oil: The fatty acid composition of the oil was determined by the GC-MS (Gas Chromatography-Mass Spectrometry) analysis (Franca Angerosa, 1995). Thermo Scientific TSQ 8000 Gas Chromatograph with the TRACE 1300 GC (Thermo Scientific Trace Finder Software), a silica column (30mm x 0.25mm id, film thickness of 0.25µm) was used for separation. GC analysis separates all of the components in a sample and provides a representative spectral output. The injector temperature and detector temperature were set at 250°C and 220°C , respectively. The sample (1.0µl) was injected into the injection port of the GC device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically. The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak.

Statistical analysis: The experimental results are expressed as mean ± standard deviation of triplicate measurements and the results were processed using Microsoft Excel 2010. The statistical analysis was performed using ANOVA to check the significance of extraction methods on different physico-chemical properties at $P<0.05$.

RESULTS AND DISCUSSION

Effect of pre-treatments on yield of groundnut oil: The oil can be extracted with various methods from the groundnut kernels. The average oil recovery from ghanis, mechanical pressing machines and solvent extraction is 30%, 45% and 47.5%, respectively (Yusuf, 2018).

In the present study, the yield of groundnut oil ranged between 35.20% and 48.14% for different treatments. The oil yield was found to be highest (48.14%) for finely ground groundnut with heat treatment (T3) and lowest for the raw kernels without heat treatment (T6) i.e. 35.20% (Figure 1). Results revealed that oil yield decreased with increasing particle size and increased with the application of heat treatment. The reason for the same is the availability of increased surface area for the easy release of oil. Similar results were reported for maize germ oil (Shende and Sidhu, 2019; Datt and Kaur, 2019); for apricot kernel oil (Gayas *et al*., 2016), and for palm fruit oil (Kasmin *et al.,* 2016). It is evident that the oil yield increased as the pressing time

increased. The maximum time for oil extraction was recorded in the finely ground samples, with and without heat treatment and the oil yield is also the highest in the finely ground sample. The minimum time taken for oil extraction (15 min) was observed in raw sample kernels with heat treatment.

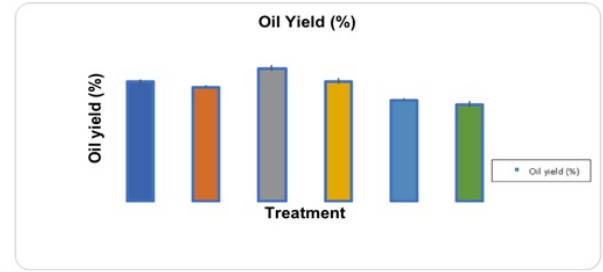

Fig. 1. Effect of different process parameters on oil yield

Effect of process parameters on quality of groundnut oil: The effect of the pre-treatments on different quality parameters of oil is presented in Table 2. The saponification value of groundnut oil ranged from 106.34 to 140.25 mg KOH/g. The maximum saponification value of 140.25 mg KOH/g was observed in the sample of oil extracted from preheated fine ground kernels (T4). Saponification value of the oil extracted from the raw kernels with heat treatment (T5) was the minimum (106.34 mg KOH/g). It is evident that the heat treated samples had lower saponification value as compared to the samples without any heat treatment. The results obtained were in accordance with those of Nkafamiya *et al*. (2010) on oils of non-conventional leafy vegetables. Higher the saponification value, greater is the percentage of the short chain acid present in the glycerides of oils and fats. Thus, lower the saponification value of oil, better is the quality of oil. Similarly, saponification value increased with the decrease in the particle size of the sample. It may be due to the more homogeneity of samples and increased surface area.

The iodine value of groundnut oil with different pre-treatments ranged from 99.24 (T5) to 111.10 mg I2/g oil (T4-oil from sample of finely ground groundnut without heat treatment). When the sample was ground, the iodine content was higher in the samples without heat treatment. In case of whole kernels, the iodine content was lower when heat treatment was given to the kernels. The iodine value is a measure of the degree of unsaturation in an oil. Iodine value is a useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid (Sadasivam and Manickam, 2008). Thus, a lower value of iodine is considered desirable for optimum quality of oil. The highest value (1.15 M eq/kg) of peroxide content recorded was in the sample of finely ground samples without heat treatment (T4). The least value of peroxide content was with the oil extracted from whole kernels sample with heat treatment and was found to be 0.65 M eq/kg (T5). Detection of peroxide content gives the initial evidence of rancidity of unsaturated fats and oils. Oils with a high degree of unsaturation are most susceptible to oxidation. Thus, a lower peroxide value is desired in the oil. It is evident that the oil extracted from whole kernels and subjected to heat treatment is of better quality as it has least peroxide value and thus would be less prone to rancidity and oxidation. Kaleem (2015) reported that the oils with higher peroxide values are more susceptible to microbial rancidity, leading to production of unpleasant odors and flavors. He observed that when oils are subjected to higher frying temperatures, the peroxide value is found to increase or stay constant and thus addition of antioxidants and preservatives in the oil is required to enhance the quality and shelf life.

The highest value of bulk density (0.64±0.01 g/ml) was recorded in the oil extracted from the sample of the finely ground kernels without heat treatment (T4), whereas, the lowest value (0.53±0.01 g/ml) was found in the sample of oil extracted from the raw kernels with heat treatment (T5), which may be due to slight decomposition of triacylglycerides during the heat treatment (Yoshida *et al.*, 2005).

Effects of various pre-treatments on the colour of groundnut oil: The effect of parameters on colour values of groundnut oil is presented in Table 3. It was observed from the table that the highest 'L*' value (27.7) was recorded in the oil extracted from the sample of raw kernels without heat treatment (T6) and was pale yellow in colour while the oil extracted from the sample of coarse grinded kernels with heat treatment (T1) had the least 'L*' value i.e. 26 while the a* and b* values were -3.2 and 5.4 respectively. The maximum and minimum chroma values were found in samples T5 (7.75) and T1 (6.27). The 'Hue' values ranged from -59.35 (T1) to -70.39 (T5). The colour of oil changed from light yellow to a darker shade of green as the 'Hue' value decreased. When heat treatment was given to coarse kernels, the colour became light yellow that adds to the desirable market value of the oil (Table 3). The heating of the samples resulted in the augmentation of hydro-peroxide which may be due to decomposition of colour components. Due to heat, size reduction of particles as well as intensity of colour increased. The colour of oil of finely ground sample of groundnut was dark green in colour because of size reduction and increased surface area for colour decomposition and the visual quality continued to decrease with reduction in size. The maximum and minimum recorded values of a* were for sample T5 (-2.6) and for sample T3 (-3.2) respectively. On the other hand, the values of b* ranged from 5.4 in sample T1 and 7.3 in sample T5.

Table 2 Quality of groundnut oil affected by various process parameters

Treatments	Saponification value (mg KOH/g)	Iodine value (mg I_2/g oil)	Peroxide value (M eq /kg)	Bulk density (g/ml)
T1	112.2±0.89	102.24±0.31	0.80±0.19	0.59±0.01
T2	129.03±0.71	106.89±0.14	0.85±0.15	0.56±0.02
T3	128.62±0.45	109.62±0.34	0.87±0.19	0.54±0.01
T4	140.25±0.71	111.10±0.26	1.15±0.18	0.64±0.01
T5	106.34±0.71	99.24±0.10	0.65±0.17	0.53±0.01
T6	109.14±0.45	100.20±0.24	0.70±0.15	0.55±0.01

Table 3 Colour value of groundnut oil

Treatments	L*	a*	b*	Hue	Chroma
T1	26±0.02	-3.2±0.06	5.4±0.11	-59.35±0.08	6.27±0.06
T2	26.5±0.01	-2.9±0.16	5.7±0.06	-63.03±0.12	6.39±0.16
T3	26.1±0.12	-3.3±0.20	5.8±0.07	-60.36±0.06	6.67±0.20
T4	26.4±0.03	-2.9±0.11	6±0.09	-60.20±0.02	6.66±0.22
T5	27.6±0.06	-2.6±0.10	7.3±0.06	-70.39±0.02	7.75±0.08
T6	27.7±0.16	-2.8±0.06	6.7±0.16	-67.31±0.09	7.26±0.02

Table 4 Effects of pre-treatment on fatty acid composition of groundnut oil

Treatments	Stearic acid content (%)	Oleic acid content (%)	Linoleic Acid (%)	Palmitic Acid (%)
T1	2.58±0.03	51.2±0.21	29.63±0.18	11.49±0.17
T2	2.43±0.27	49.61±0.48	31.2±0.05	11.11±0.31
T3	2.73±0.40	50.46±0.62	30.52±0.10	11.15±0.13
T4	2.61±0.03	49.56±0.62	31.44±0.34	11.03±0.13
T5	2.88±0.36	51.39±0.33	30.02±0.21	12.07±0.38
T6	2.99±0.05	50.04±0.47	29.05±0.18	12.22±0.39

Table 5 (a) ANOVA of effect of treatments on physico-chemical properties of groundnut oil

Properties	d.f.	M.S.	F	P-value	F critical
Iodine Value	5	71.136460	71.85	0.013783*	3.33
Peroxide Value	5	0.98827300	529.88	0.001885*	3.33
Saponification Value	5	552.90830	27947.91	0.000036*	3.33
Bulk Density	5	0.62856670	43.17	0.022794*	3.33

Table 5 (b) Significance of treatments on physico-chemical properties of groundnut oil

Treatments	Iodine Value	Peroxide Value	Saponification Value	Bulk Density
T1	104.066*	0.806	112.200*	0.586
T2	106.800*	0.850	129.033*	0.550
T3	109.600*	0.856	128.600*	0.546
T4	111.133*	1.183*	140.066*	0.656
T5	99.300	0.663	106.333	0.533
T6	100.166	0.720	109.033	0.550
CD (5 %)	1.81	0.25	0.26	0.22

* Significant at P=0.05

Effects of pre-treatments on fatty acid composition of groundnut oil: Fatty acids make up the major portion of the weight of an oil molecule, the physical and chemical properties of the oil tend to be determined by properties of the fatty acids predominating in their make-up (Aruna and Nigam, 2009). Although up to 12 fatty acids have been reported in groundnut, generally palmitic acid (16:0) constitutes nearly 10% and the oleic (18:1) and linoleic acid (18:2) proportions together make up 80% of the fatty acid composition in groundnut (Ahmed and Young, 1982; Ahmad and Mirani, 2012). In the present study, the estimated values of fatty acids were comparable within the treatment and their composition was not influenced by different pre-treatments (Table 4).

Statistical analysis of effect of pre-treatments on quality of oil: The data obtained were subjected to Analysis of Variance [Table 5 (a)]. The statistical analysis at 5% level of significance ($P<0.05$) indicated that both particle size of kernel and heating pretreatment had significant effects on all the properties of oil *viz;* iodine value, peroxide value, saponification value, bulk density. The iodine value determines the amount of unsaturation in fatty acids. In the present study (Table 5b), the iodine value of the treatments T4 (fine kernels without heat treatment) was significantly higher than the control sample (T6), however, the treatment T5 was at par with the control sample. Similar trends were also obtained for peroxide value, saponification value and density of oil. Overall, pretreatment indicated by T5 i.e. whole kernels with heat treatment was found to be the best among all the pretreatments with better retention of physico-chemical properties of oil.

The present research assessed the effects of particle size and heat treatment on recovery and quality of groundnut oil. It could be concluded that the coarseground kernels followed by heat treatment (60°C for 1 h) resulted in 43.33% of oil having best quality in terms of colour, fatty acid composition, longer shelf life and resistance to rancidity and oxidation. The oil thus extracted had maximum value of oleic and linoleic acid content of 51.39 % and 31.44% respectively.

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Soybean Gyan - a mobile application for effective soybean knowledge dissemination

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ABSTRACT

Soybean Gyan Mobile App is developed for soybean growers to take right decisions in the real-time field conditions. It contains comprehensive knowledge on soybean crop management on one platform. The design and development of the mobile App is discussed in the paper. The salient features of the App are presented in detail. The functionality of the App and overall look and feel of the App is given. The app is available for free download from Google play store. It is a user friendly App that can cater to the needs of all the stakeholders like farmers, agricultural advisors, agriculture students or research scholars and line department officials. This app is being used by many as indicated by the number of downloads (over 10000) within a short time.

Keywords: Crop Management, Mobile App, Soybean, Soybean Gyan

Among the major soybean growing countries, India ranks fourth in terms of area and fifth in terms of production as per USDA estimates while as per DAC estimates it ranks fourth both in terms of area and production. Soybean contributes 47% of the total oilseeds and about 26% of the total edible oil produced in the country. Total area under the crop is 12.5 million hectares accounting for 35.89 million tons of soybean grain production in the country (AICRPS Report, 2019-20). Despite extraordinary growth in area and production of soybean during the past 40 years, the current productivity levels are much below the world average.

In this digital era, with the advancement of technologies, storage of large volume of data and accessing information at high speed has become effortless and affordable even though remote area still faces the problem in accessing the information. Technology helps to gain the right information at right time and right place. Software reduces the efforts of researcher, farmers and it automates the tedious task. Software helps to access data in a systematic manner to avoid confusion between relative data.

Estimates indicated that 60% of farmers do not have access to any source of information for advanced agricultural technologies resulting in huge adoption gap. Government had launched some apps to help farmers to get complete information of the crops. But all the information is not available in a single app. Moreover the information resources available in cyber world, have very limited information on soybean crop. Most of the popular apps developed for agriculture are generalized covering many crops i.e., they are not specific to soybean (Plantix, 2015; Kisan Suvidha, 2016; Pusa mKrishi, 2016; Madhuri *et al.,* 2018; Madhuri *et al.,* 2020). A few apps available for soybean crops (Soya Guru, 2020; Soybean-Insecticide & Fungicide calculator, 2018; Soybean Disease app, 2011; Soybean cultivation, 2017) do not have comprehensive information about all the technological aspects in a single app.

Therefore, the mobile App-Soybean Gyan developed for soybean growers has the potential to provide the needed impetus to soybean sector. Soybean Gyan can complement the traditional extension system for dissemination of soybean technical knowledge to the stakeholders across the country. The App is developed in Hindi and English languages for the ease of access to all the stakeholders. The development of Hindi version of Soybean Gyan is discussed in the paper.

MATERIALS AND METHODS

Soybean Gyan Mobile App was developed using Android Studio that is an open source App development platform. The App is available free of cost at Google Play store (Fig. 1). The users can download and install the App on their Android mobile phones having at least 11 MB free memory. The App integrates following modules on a single platform to help farmer community.

- Agronomical practices, crop production and production techniques
- Disease Management
- Insect Management
- Weed Management
- Health benefits and home use with soy food recipes
- Seed treatment and storage
- Farming Equipments
- Important Facts

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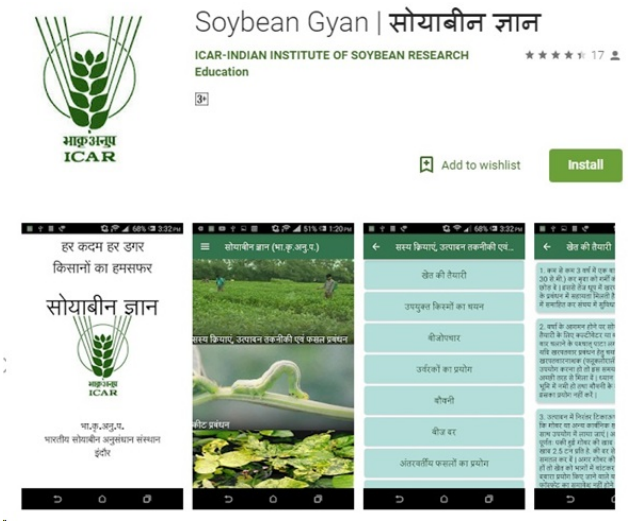

Fig. 1.Soybean Gyan on Google play store

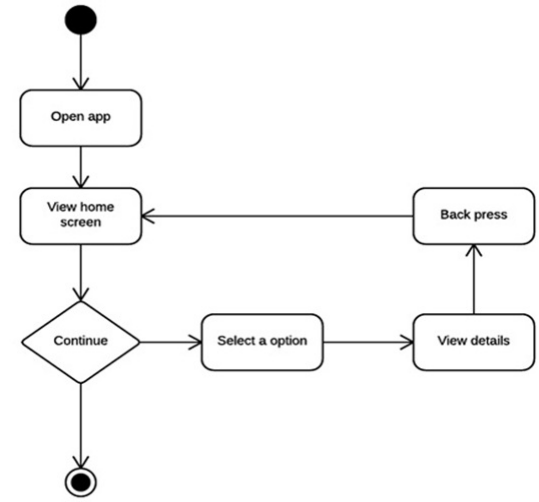

Fig. 2. Activity diagram of user

The design and developmental details of the App are discussed below.

Hardware requirements: The App works smoothly on any Android device having Android version 4.0 and higher with at least 11 MB memory size.

System flow: The user first installs the App on his Android phone. The user then opens the App and selects the menu options given on the screen to get the desired information. The overall flow of user control can be seen in Fig. 2. The user will open the App, view the screen for different options available, he will select the option as per his need and view the detailed information required by him.

AndroidManifest.xml file: Soybean Gyan android application has an AndroidManifest.xml file in its root directory. This file lists out all the activities, intents, intent-filters, permissions etc. for the application use. This file is responsible for providing all the information about the application to the Android system. The part of the code of AndroidManifest file is shown in Fig. 3.

Activities: Soybean Gyan App works by invoking different activities. The left panel in Fig. 3 shows the list of Activities involved in the Soybean Gyan App development. An Android activity is one screen of the Android app's user interface. An activity provides a means of interaction to the user. An Android app may contain one or more activities, i.e one or more screens. The Android app starts by initiating the main activity, and from there the app may make it possible to open additional activities. The details of the different Activities used in the Soybean Gyan App are shown in Table 1 below.

Activity lifecycle -Whenever user starts using Soybean Gyan mobile app, Main activity, is first launched and user sees the first screen with menu options. A particular Activity is launched, based on the choice of the user on the main screen. The user gets the information based on his selection on the main screen for which another activity is launched.

The complete Activity Lifecycle is depicted in the Fig. 4. (Android App, 2020)

There are four stages of an activity in Soybean Gyan Mobile App.

i) Activity Launched- It is a stage when an activity is initiated or started.

ii) Activity Running- If an activity is in the foreground of the screen, then it is said to be active or running. This is usually the activity that the user is currently interacting with.

iii) Activity Shutdown or Stop-If an activity is completely hidden by another activity, it is stopped or hidden.

iv) Activity Kill - The system destroys the activity from memory by asking it to finish or simply killing its process.

Each activity goes through various stages or a lifecycle. There are seven stages of an activity.

i) onCreate - It is a stage when activity is first created.

ii) onStart - It is a stage when activity is becoming visible to the user.

iii) onResume - It is a stage when activity will start interacting with the user.

iv) onPause - It is a stage when activity is not visible to the user.

v) onStop - It is a stage when activity is no longer visible to the user.

vi) onRestart - It is a stage after an activity is stopped, prior to start.

vii) onDestroy - It is a stage before the activity is destroyed.

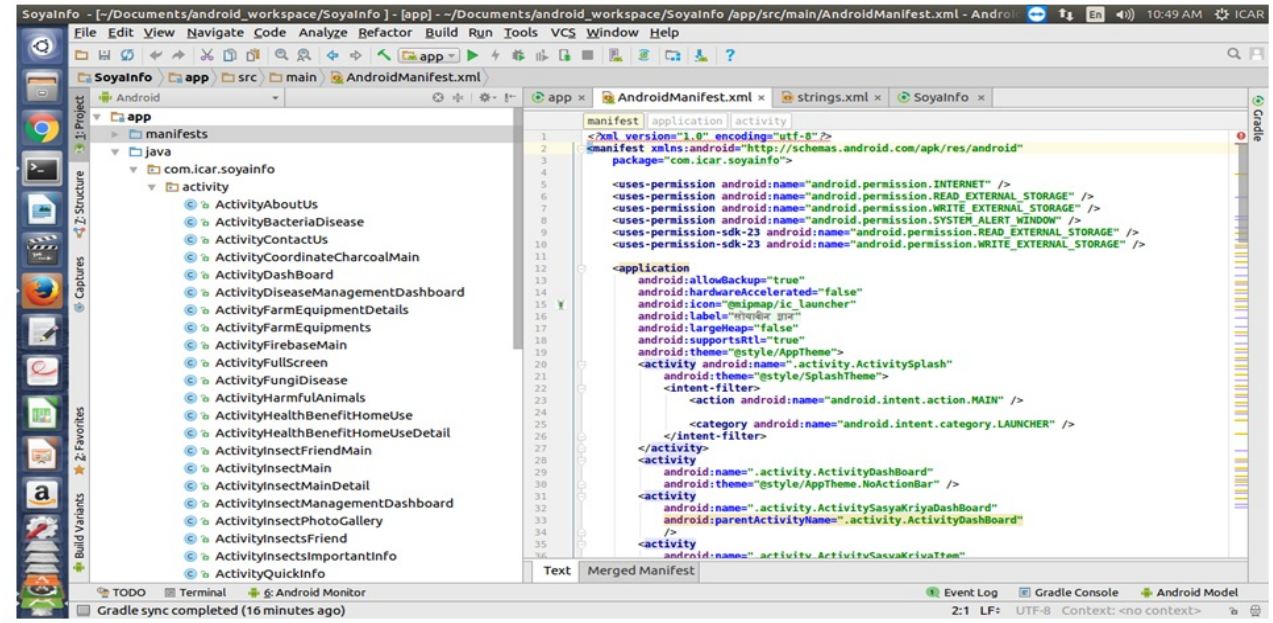

Fig. 3. Source code of AndroidManifest.xml file of Soybean Gyan App

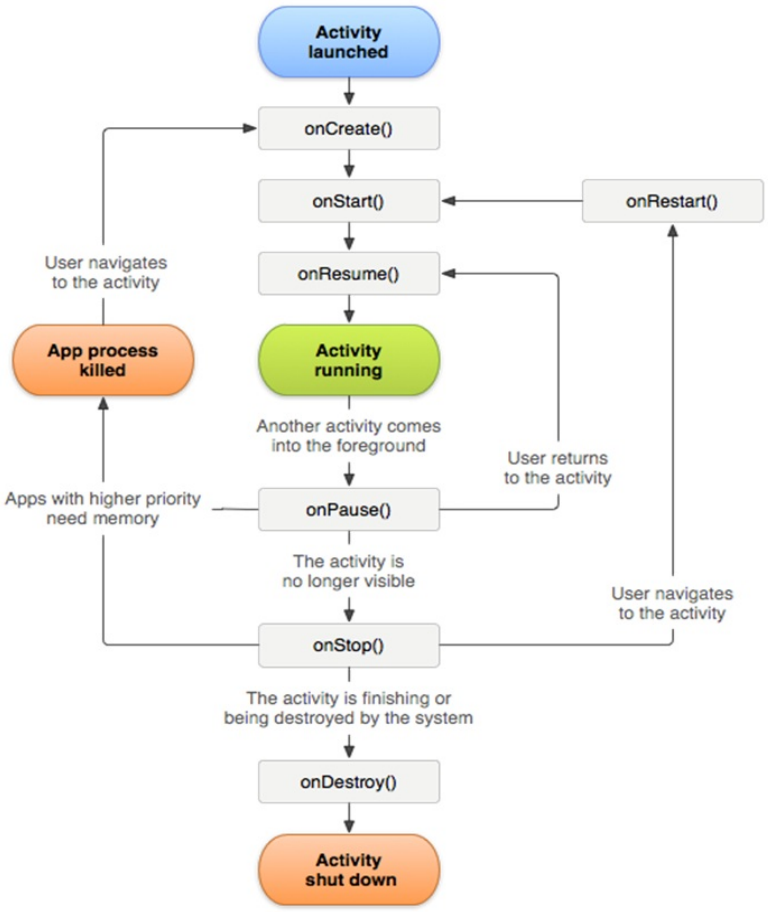

Fig. 4. Activity Lifecycle of Soybean Gyan Mobile App

Table 1 Different activities used in the Soybean Gyan App and its details

Activity	Details
ActivityDashBoard	Main menu-option interface.
ActivitySasyaKriyaDashBoard	User Interface to get information about agronomy practices and information on soybean crop management.
ActivitySasyaKriyaItem	
ActivitySasyaKriyaPrabhandhan	
ActivityHealthBenefitHomeUse	Interface for details about soybean health benefits and its food uses.
ActivityHealthBenefitHomeUseDetail	
ActivitySoybeanKeUpyog	Information on Soybean recipes.
ActivityWeedManagementDashboard	Interface for Soybean Weed Management and its control.
ActivitySoybeanWeedManagement	
ActivityWeedManagementPrecautions	
ActivityDiseaseManagementDashboard	Interface for Soybean Disease details-Bacterial, Fungal and Viral and disease management practices.
ActivityBacterialDisease	
ActivityFunglDisease	
ActivityVirusDisease	
ActivityFarmEquipments	Interface for Farm Equipments details.
ActivityFarmEquipmentDetails	
ActivityInsectManagementDashboard	Interface for Soybean Insect details and Management. It provides information on integrated pest management practices, friendly insects, insect related important facts and insects' photo gallery.
ActivityInsectMain	
ActivityInsectMainDetails	
ActivityInsectFriendMain	
ActivityInsectsFriend	
ActivitySamekitKeetPrabhandhan	
ActivityInsectPhotoGallery	
ActivityInsectsImportantinfo	
ActivityQuickInfo	Interface for quick access to important information on all technological aspects.
ActivityContactUs	Contact details of domain experts and officials.
ActivityAboutUs	About the sources of information and institute information.

The functionality of the App and overall look and feel of the App is discussed in detail in the section below.

RESULTS AND DISCUSSION

The App provides information on - i) Agronomic Practices, Production technology and Crop management, ii) Insect Management, iii) Disease Management, iv) Weed Management, v) Health Benefits and Food Uses, vi) Farm Machinery and vii) Important information for Soybean cultivation (Fig 5).i)

Agronomic Practices, Production technology and Crop management: It has information about field preparation, selection of suitable varieties, seed treatment, use of fertilizer, sowing, seed rate, intercropping, water management, harvesting and seed storage (Soybean Production, 2019; Soybean Unnat Prajatiyan, 2019).

Insect management: It has information about major insects, integrated pest management, soya friendly insects and other important information about insect damage and losses. Presently it contains detailed information about twenty most damage causing insects. The insect information includes description about appearance of insect and its behavior, losses caused and its appropriate management (Soybean Samekit Keet Prabandhan, 2019).

Disease management: It contains information about most prevalent soybean diseases, its symptoms and its effective management. The diseases are categorized into three categories-Fungal, Bacterial and Viral. The user gets details on causal organism, general description, symptoms and recommended management practices (Soybean Samekit Rog Prabandhan, 2019).

Weed management: It includes complete details of different types of weeds observed in soybean fields, losses caused by them, recommended weed management practices, integrated weed management package of practices and important precautionary measures for effective weed management in soybean. It also provides information on recommended doses of different weedicides to control different types of weeds in soybean fields (Soybean Samekit Kharpatwar Prabandhan, 2019).

Health benefits and food uses: It has information on health and nutritional benefits of soybean usage at household levels. The health benefits are described in context to the diabetes, cancer, osteoporosis, heart disease and menopausal problems. It also has information on soybean food recipes *viz.,* Soy Milk, Soy Paneer (Tofu), Soya flour, Soybean Namkeens, Soy toffee etc. (Soybean Prasanskaran Takniki evam Khadya Upayog, 2019).

Farm machinery: It includes detailed information for farm machinery used for cultivation viz., sub-soiler, broad bed furrow machine, furrow irrigated raised bed system machine and ridged fertilizer drill cum seed planter. It has details on salient features of the machine, cost and the method of procurement.

Important information for Soybean cultivation: It includes information on month-wise agricultural practices, recommended technologies, recommended insecticides and weedicides. (Soybean Production: Package of Practices and Technical Recommendations, 2020).

In addition to this, Soybean Gyan App also has a Navigation drawer (Fig. 6) for direct links to this information. It also has links to the decision support system for insect management in soybean, farmer query system, weekly farmer advisory and contacts to the domain experts to solve specific field problems. The App can be freely downloaded from the link https://play.google.com/store/apps/details?id=com.icar.soyainfo&hl=en from the Google play store. Based on the feedback from the users of the app, Soybean Gyan is highly useful and a good source of complete soybean crop management information. This is proved from the average review ratings as 4.2 as on date and good review comments written on Google play store.

Therefore, Soybean Gyan Mobile App is a rich source of information for soybean crop management to all the stakeholders engaged in soybean value chain. Soybean growers are showing keen interest that can be seen from the fact that it has more than ten thousand downloads till date on Google play store. The users of the app, found it as highly useful and a good source of complete soybean crop management information. This is indicated by the average review ratings as 4.2 and good review comments submitted by users on Google play store.

Fig. 5. Important information provided by the App for soybean cultivation

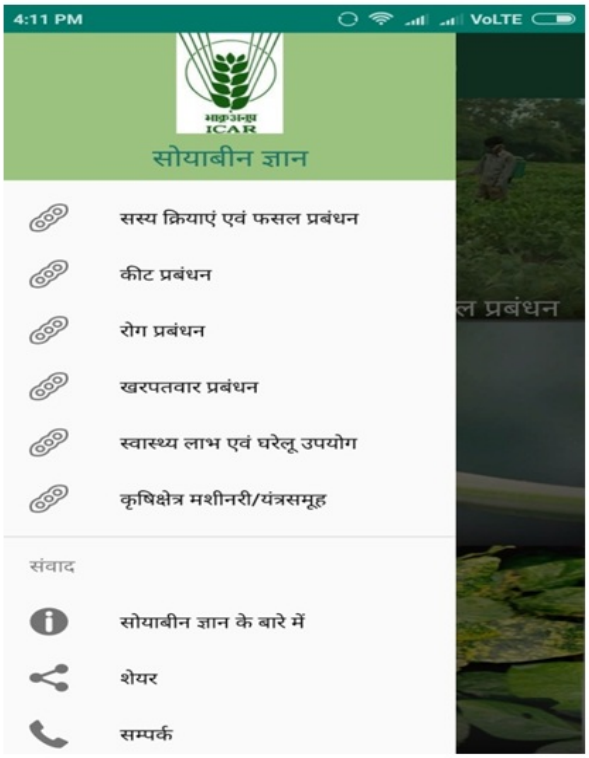

Fig 6 Navigation drawer having direct links to all information

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Screening of linseed (*Linum usitatissimum* L.) germplasm under epiphytotic conditions against major foliar diseases

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ABSTRACT

A set of 258 linseed germplasm accessions were evaluated for major foliar diseases under epiphytotic conditions at AICRP linseed experimental block, Main Agricultural Research Station, Raichur during *rabi* 2019-20. Among the tested germplasm none of the lines were completely free from powdery mildew and Alternaria blight infection. The germplasm accession 20204 showed resistance for powdery mildew but no resistant source is recorded against Alternaria blight. Under moderately resistant group, 19801 and 20216 showed resistance to both Alternaria blight and powdery mildew. Twenty germplasm lines were identified against powdery mildew disease. The rest of the germplasm were moderately susceptible, susceptible or highly susceptible to powdery mildew and Alternaria blight infection.

Keywords: Alternaria blight, Germplasm, Linseed, Powdery mildew, Resistant

Linseed (*Linum usitatissimum* L.) is known as a founding crop which serves as a crop platform for the production of bio-industrial and nutraceutical products (Jhala *et al*., 2008). It is one of the oldest cultivated and the 6th largest oilseed crop in the world. (Bhatty and Rowland, 1990; Kumari *et al.,* 2018). The crop is native to west Asia and the Mediterranean. Linseed is also known as 'Flaxseed', an annual dicotyledonous plant which belongs to the family Linaceae and grown during *rabi* season crop as either oil crop or a fibre. Canada is the world's largest producer of flax (38 % of total production). India contributes about 14.88 % and 6.57 % to the world area and production, respectively. Productivity of Rajasthan state (1114 kg/ha) of India is surpassing the productivity of Asia (728 kg/ha) as well as of the world (986 kg/ha) (Anonymous, 2019; Tewari and Singh, 2018).

Linseed is one of the richest sources of α-linolenic acid (ω-3 fatty acid) and soluble mucilage. An analysis of brown Canadian flax showed about 41 % fat, 20 % protein, 28 % total dietary fibre, 7.7 % moisture and 3.4 % ash, which is the mineral-rich residue left after samples are burnt (Hurteau, 2004). In general, the seed contains 20 % protein but Indian cultivar Khategaon had a protein content of 21.9 % and is free from gluten. Linseed oil have omega-3 (ω-3) (57 %), omega-6 (ω-6) (16 %), monosaturated fatty acid (18 %) and saturated fatty acid (9 %) in its composition (Katare *et al.,* 2012). The components present in flaxseed attract the food technologists and nutritionists to explore its activities in health sector. Linolenic acid, eicosapentaenoic acid (EPA) -- and docosahexanoic acid (DHA) are the three types of ω-3 fatty acids and are nutritionally important because they reduce the risk of cardiovascular disease. Flaxseed can be incorporated into diet through oil, milled or ground flaxseeds or through eggs, meat produced by animal fed flax meal. The seeds are now widely used in bakery (Fitzpatrick, 2007). One tablespoon of ground flax per serving can be incorporated into morning hot cereal after cooking. We can also sprinkle ground flax over salads, cooked vegetables or cold breakfast cereals.

Linseed is adversely affected by different diseases. Out of different fungal diseases of linseed, most important pathogens are *Alternaria lini* (blight), *Fusarium* spp. (wilt), *Botrytis cinereal* (gray mould) and *Oidium lini* (powdery mildew) (Mercer *et al.,* 1991) or *Leveillula taurica* (Arshiya *et al.,* 2017; Ajithkumar *et al.,* 2017), *Ascochyta linicola* (foot rot), *Melampsora lini* (Rust), *Rhizoctonia solani* (Rhizoctonia seedling blight), *Pythium megalacanthum* (scorch), *Septoria linicola* (pasmo), *Polyspora lini* (browning or stem break) and *Colletotrichum linicolum* (anthracnose).

Among the foliar diseases powdery mildew is the most serious and devastating foliar disease in Northern Karnataka. A white powdery, dust like coating on leaves, stems as well as the pods with mycelium and spores of the fungus characterize the disease. This disease is more prevalent in late sown crop, where the severity can progress up to 60% (Ajithkumar *et al.,* 2014; Arshiya *et al.,* 2016). Alternaria blight is a major disease which causes heavy loss in terms of quality and quantity of fibre as well as seed of linseed. Alternaria blight was first reported by Dey (1933) from Kanpur, Uttar Pradesh in 1933 and fungus was identified as *Alternaria lini*. Later Siddiqui (1963) reported the

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occurrence of Alternaria blight on linseed cultures at IARI, New Delhi and other parts of the country. Alternaria blight causes heavy loss from 28 to 60% depending upon the variety/genotype and date of sowing. Apart from these two diseases phyllody has also been identified in linseed crop from the Raichur centre during 2014-15. Hence, an effort has been made to screen the germplasm against phyllody disease.

Minimization of chemicals for the management of diseases in crop is essential for the environmental safety. Genetic resistance is a good way to achieve this. Therefore, it is essential to evaluate large number of available germplasm lines for seed yield along with the prevailing diseases of the area. Among the various management mechanisms studied, host-plant resistance is supposed to be the best and widely explored strategy. It focuses on inhibition of fungal colonization and/or toxin production by fungus on the host plant. The utilization of such resistant varieties has been the hope for developing resistant genotypes (Ajithkumar *et al.,* 2018b). Therefore, there is a need to develop varieties resistant to multiple diseases along with stabilization of the yield potentials. The manipulation of inherent potential of plants in the form of resistant varieties is a cheap, viable and environment friendly alternative to reduce losses from biotic stress. In view of this, the present research work encompassed evaluating 258 lines for the severity of powdery mildew and Alternaria blight incidence to identify the resistant sources to the multiple diseases which could be used as resistant donor in linseed breeding programme.

A field experiment was conducted to screen 258 germplasm of linseed crop which constitutes the different parts of the country under AICRP linseed trials to identify the resistant sources for the multiple diseases. All the tested germplasm were sown during the first fortnight of November in order to make the favourable weather conditions coincide with the crop development to achieve maximum disease severity. The germplasm were sown with 45 cm spacing between the lines and 10 cm spacing between the plants and to facilitate the good inoculums build up, susceptible check (Chambal) was grown after every 10 germplasm lines and to assess the resistance reaction, resistant check (Sheela) was also repeated after every 10 entry lines. The experiment was taken up at AICRP (Linseed) experimental block, Main Agricultural Research Station, University of Agricultural Sciences, Raichur during *rabi* 2019-20. The centre is identified as hot spot for powdery mildew disease and thus the screening is effective. The recommended package of practices was followed to raise a good crop. The intensity of powdery mildew and blight disease in the field was estimated from five randomly selected plants in each genotype which were tagged with labels at the flowering stage of the crop. On an average 10 leaves were selected at random from the plants and disease severity was recorded by visually examining each leaf and the disease severity was scored using 0-5 scale as described (Anonymous, 2019).

Further, the % disease index was calculated by using the formula given by Wheeler (1969).

$$\text{Sum of individual ratings} = \frac{\text{Per cent Disease Index}}{\text{No. of plants examined X Max. disease scale}} \times 100$$

The screening trial revealed that, none of the tested lines was completely free from powdery mildew and Alternaria blight infection. However, significant variations in disease severity index for powdery mildew and Alternaria blight were observed among the tested lines.

Only one germplasm namely 20204 showed resistant reaction against powdery mildew disease but none of the germplasm was resistant to Alternaria blight infection. About 22 lines (KL-219, KLSD-10, NDL-2009-21, NDL-2005-26, RL-26016, SLS-61, LBR-6, KLS-C-8, 19801, 19807, 19810, 19839, 19843, 20207, 20209, 20216, 20220, 20222, 20229, 20232, 20245 and 20248) were grouped as moderately resistant for powdery mildew, and the group also included two lines, 19801 and 20216, which were moderately resistant for Alternaria blight infection. The present study revealed that only two germplasm namely 19801 and 20216 were moderately resistant to powdery mildew and Alternaria blight infection.

Table 1 Disease severity rating for screening of linseed germplasm against powdery mildew and Alternaria blight infection

Rating	Description	Reaction
0	No disease or free	Highly Resistant (HR)
1	0 to 10 per cent area of leaves/plant infection	Resistant (R)
2	10.1 to 25 per cent area of leaves/plant infection	Moderately Resistant (MR)
3	25.1 to 50 per cent area of leaves/plant infection	Moderately Susceptible (MS)
4	50.1 to 75 per cent area of leaves/plant infection	Susceptible (S)
5	Above 75 per cent area of leaves/plant infection	Highly Susceptible (HS)

Table 2 Reaction of linseed germplasm against major foliar diseases of linseed during *rabi* 2019-20

Disease reaction	Name of the germplasm	
	Powdery Mildew	Alternaria Blight
Highly Resistant (HR)	Nil	Nil
Resistant (R)	20204 (01)	Nil
Moderately Resistant (MR)	KL-219, KLSD-10, NDL-2009-21, NDL-2005-26, RL-26016, SLS-61, LBR-6, KLS-C-8, 19801, 19807, 9810, 19839, 19843, 20207, 20209, 20216, 20220, 20222, 20229, 20232, 20245, 20248 (22)	19801, 20216 (02)
Moderately Susceptible (MS)	78	28
Susceptible (S)	43	108
Highly Susceptible (HS)	12	17

The present results are based on the screening of germplasm under natural epiphytotic field conditions. Environmental factors such as humidity and temperature have greater influence on the development of the disease possibly explaining these differences in their differential reactions. Therefore, the resistant genotypes need to be evaluated under artificial conditions to confirm the resistance before using them in breeding programme. Narender and Tripathi (2018) screened 200 genotypes of linseed against Alternaria blight, of which none was disease-free or highly susceptible, seven genotypes were resistant, 66 were moderately resistant, 102 were moderately susceptible and 25 were susceptible against blight disease of linseed. Ajithkumar *et al.* (2015) screened a broad range of 371 linseed germplasm against powdery mildew which revealed that 17 germplasm (EC-41656, FR-3, Kanpur - 41/2, GS-232, LS-35, LCK-11, POLF-16, POLF-17, OR-1-4, S-801, JRF-1(8), S-91-26, RL- 903, Meera, EC-322646, UDN-55, IDSN-6) were highly resistant even after artificial inoculation, whereas 16 germplasm showed symptoms after the inoculation but the infection was not severe and recovery was rapid. The present work is in agreement with Dash *et al.* (2016).

The success of breeding programme is primarily dependent on the genetic variation in the breeding material. Evaluation and understanding the scope of genetic deviation existing in the germplasm is important and leads to effective deployment of the germplasm thus only a small portion of genetic variability has been exploited in genetic enhancement of the crop. The variability in the germplasm is the primary source of resistance to the disease. Plant breeders need to explore the nature of disease resistance and identify additional resistance genes from new sources. Hence effort made in this research work helps in developing varieties resistant to powdery mildew to stabilize the yield potentials of linseed varieties.

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Yield, nutrient uptake and economics of Indian mustard (*Brassica juncea*) influenced by nutrient doses and planting geometry under new alluvial zone of West Bengal

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ABSTRACT

Field experiments were conducted during 2017-19 to study the effect of different doses of nutrients and planting geometries on the performance of Indian mustard under new alluvial zone of West Bengal. The field experiment was conducted in split plot design with three replications, having twenty treatment combinations of five nutrient doses in main plot and four crop geometries in subplot. Seed yield (1751 kg/ha) of Indian mustard with 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively (125 % RDF) was at par with 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively i.e. 100% RDF (1674 kg/ha) and 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively i.e. 150 % RDF (1733 kg/ha) and significantly higher than 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively i.e. 75 % RDF. Application of 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively registered 42.5 % more stover yield over 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively and was significantly superior to all lower doses of nutrients. Application of 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively resulted in higher oil content than other nutrient doses. Highest protein content obtained with the 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively was significantly higher than 30:20:20 kg N, P_2O_5 and K_2O/ha, respectively and 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively. Highest oil and protein yield registered with application of 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively were at par with the 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively and 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively for oil yield and 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively for protein yield. Economics revealed that application of 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively gave maximum net return (₹ 43,250/ha) and B:C ratio of 3.01. The highest seed yield obtained with the 25 x 20 cm spacing (1644 kg/ha) was significantly higher than all other treatments of planting geometry. This treatment registered 24.2 % more seed yield over 25 x 15 cm planting geometry. Plant spacing of 25 x 20 cm registered more stover yield and showed parity with 30 x 15 cm spacing. Closer spacing (25 x 20 cm and 25 x 15 cm) resulted in significantly higher oil content over all other crop geometries. Planting geometry of 25 x 20 cm registered highest oil yield and was comparable only with 30 x 15 cm. However, highest protein yield observed with the 30 x 30 cm spacing was on par with 25 x 20 cm and 30 x 20 cm plant spacing. Plant spacing of 25 x 20 cm gave highest net return (₹43,260). However, maximum benefit: cost ratio (3.51) was recorded with the plant spacing of 30 x 15 cm.

Keywords: Mustard, Nutrient, Oil, Protein, Spacing, Yield

Oilseeds which share about 14.1 per cent of the total cropped area in India are next to cereals and play an important role in agricultural economy of the country. Rapeseed-Mustard (*Brassica* species) is an important group of crops among oilseeds and comprises Indian mustard, Indian rape, oilseed rape, Ethiopian mustard, taramira, and black mustard. India ranks first in area and second in production of rapeseed-mustard after China in the world. Rapeseed-mustard group of crops account for 3 per cent of total cropped area in India and contribute 28.6 per cent to total production of oilseeds (Mukherjee, 2015). India contributes 12 per cent to world's total production of rapeseed-mustard. Yield needs to be stepped up significantly in order to increase the production of oilseeds to meet the growing demand. Among different crops of rapeseed-mustard, Indian mustard is the most adapted crop in the Indo-Gangetic zones (Singh *et al.,* 2009). The competitive ability of a rapeseed-mustard plant depends greatly upon plant density per unit area and soil fertility status (Shekhawat *et al.,* 2012). Planting geometry affects canopy structure of crops and influences light interception and radiation use and consequently growth and productivity. A uniform distribution of plants per unit area is a prerequisite for yield realization (Mukherjee, 2014) as it influences use of nutrients, moisture and suppression of weeds. In wider row spacing, solar radiation falling within the rows gets wasted particularly during the early stages of crop growth whereas in closer row spacing upper part of the crop canopy may be well above the light saturation capacity but the lower leaves remain starved of light and therefore have poor photosynthesis. At present very scanty information is available for cultivation practices of Indian mustard in new alluvial region of West Bengal which represents flood plain areas along the river courses. It has more sand and silt than clay. The present investigation was, therefore, carried out to optimize the nutrient requirements and planting geometry for Indian mustard grown in new alluvial zone.

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The experiment was conducted during *rabi* season of 2017-18 and 2018-19 under new alluvial zone at seed farm in Bidhan Chandra Krishi Viswavidyalay, Kalyani, West Bengal. The soil of the experimental field was sandy loam in texture, neutral in reaction (pH 7.1), medium in organic carbon (0.43%), available N (231.1 kg/ha), P_2O_5 (20.1 kg/ha) and K_2O (189.5 kg/ha). The total rainfall recorded during crop growth period was 17.3 and 13.5 mm, minimum temperature ranged from 12.1°C to 16.3°C and 11.9°C to 17.8°C, and maximum temperature from 22.2°C to 34.4°C and from 18.7°C to 36.3°C during winter 2017-18 and 2018-19, respectively. The field experiment was conducted in split plot design with three replications with 5 doses of nutrients (30:20:20; 45:30:30; 60:40:40; 75:50:50 and 90:60:60 kg N, P_2O_5 and K_2O/ha) in main plots and 4 treatments of planting geometry (25 x 15, 25 x 20, 30 x 15 and 30 x 20 cm) in subplots. The recommended dose of nutrients (RDF) for Indian mustard comprised 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively. Nutrients were supplied through urea, single superphosphate and muriate of potash. Indian mustard cultivar 'Giriraj' was sown on 9th October in 2017 and 10th October in 2018. Full amount of phosphorus and potassium and half amount of nitrogen as per treatments was applied at the time of sowing, while the remaining dose of nitrogen was top dressed at the pre-flowering stage after first irrigation (23 days after sowing). All other recommended agronomic practices were adopted during the crop growth period in both the years. Plant height and leaf area at peak growth stage i.e. 60 days after sowing (DAS) were recorded from five randomly selected plants from each plot and averaged. Plant height, branching, number of siliquae/plant were recorded at harvest (120 DAS) from five randomly selected plants. The seed and stover yield was computed from the yield obtained from net plot (5 x 4 m^2) and expressed in kg/ha. Initial soil status (0-15 cm) and plant uptake at harvest for nitrogen, phosphorus and potassium were determined as per standard laboratory procedures (Jackson, 1973). Sulphur uptake in plant was analyzed by turbidimetric method (Williams and Steinbergers, 1959). Oil per cent in the Indian mustard seed was determined by Soxhlet apparatus using petroleum ether (60-80ºC) as an extractant (A.O.A.C., 1960). Statistical analysis of the pooled data of growth, yield attributes and yields were performed by applying the technique of analysis of variance (ANOVA) prescribed for split plot design to test the significance of difference among treatments and conclusions were drawn at 5 % probability level. Cost of cultivation (/ha) was calculated considering the prevailing charges of agricultural operations and market price of inputs involved. Gross returns were obtained by converting the harvest into monetary terms at the prevailing market rate. Benefit: cost ratio (B:C) was obtained by dividing the gross income with cost of cultivation.

Application of 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively (100% RDF) significantly increased the plant height, dry matter accumulation, leaf area index at 60 DAS, number of primary and secondary branches/plant, seeds/siliqua, 1000 seed weight, seed yield and stover yield over lower doses of nutrients (30:20:20 and 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively). Increase in plant height with application of 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively and number of siliquae/plant, seeds/siliqua and stover yield with application of 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively over 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively was also significant. Leaf area index, number of secondary branches/plant, 1000 seed weight and seed yield increased up to 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively but such increases were non-significant over 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively. The marked improvement in growth with higher doses of nutrients could be ascribed to more response of plant to nutrient availability, which helped to exploit available resources for growth and development. The improvement in growth (plant height, number of primary and secondary branches/plant) and yield parameters with increased nutrient doses might be due to the enhanced availability of nutrient to the plant. Similar results were reported by Ram *et al.* (2003) and Kumar *et al.* (2001).The positive response of higher levels of nutrients on yield attributes could be ascribed to overall improvement in crop growth enabling the plant to absorb more nutrients and moisture and accumulate more quantities of photosynthates (Tripathi *et al.,* 2010; Rana *et al.,* 2005).

Application of 75:50:50 and 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively increased the seed yield by 48.0 and 41.5 % over 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively. The seed yield is the cumulative sum of all the yield components. Therefore, improvement in yield components significantly enhanced the seed yield. Similarly, substantial increase in seed and stover yield of mustard due to nitrogen application has also been reported by Mandal and Sinha (2004). Since phosphorus is a constituent of nucleic acid, phytin and phospholipids, enzymes responsible for transformation of energy, carbohydrates and fat metabolism, its increased uptake resulted in better growth and increased yield (Chouksey *et al.,* 2016). Similar results were reported by Mandal and Sinha (2004). Potassium is involved in carbon assimilation, photosynthesis, starch formation, translocation of protein and sugar, entry of water into plants, root development. Increased growth attributes under adequate potassium supply was responsible for better performance of yield attributes which increased with higher rate of fertilizer consisting of higher levels of potassium (Mukherjee, 2016). Stover yield with 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively (6081 kg/ha) was significantly higher than all other treatments (Table 1). Harvest index in general decreased with increasing dose of nutrients.

Application of nutrients failed to influence the N content in stover, P and K content in both seed and stover and S content in seed (Table 2). Successive doses of nutrients up to 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively significantly increased the N content in seed. Total uptake of NPK and sulphur increased with application of 90:60:60 kg/ha of N, P_2O_5 and K_2O, respectively. However, such increases were significant up to 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively for seed and up to N, P_2O_5 and K_2O, respectively for stover. Oil content decreased with successive increase in dose of nutrients from 45:30:30 kg N, P_2O_5 and K_2O/ha, respectively to 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively and protein content increased with each successive dose of nutrients up to 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively (Table 3). The reduction in oil content and increase in protein content with increase in nutrient doses might be due to the utilization of carbohydrates in protein synthesis (Kumar *et al.* 2001, Ram *et al.,* 2003, Singh and Singh, 2015). Increase in protein content was due to higher nitrogen content which is precursor of protein synthesis in seed. The highest oil and protein yields registered with the 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively were at par with the 60:40:40 kg N, P_2O_5 and K_2O/ha, respectively and 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively for oil yield and 90:60:60 kg N, P_2O_5 and K_2O/ha, respectively for protein yield. Application of 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively gave maximum net return (43,250 / ha) with B:C ratio of 3.01 (Table 3).

Planting geometry of 30 x 15 cm registered significantly more plant height, number of siliquae/plant and seeds/siliqua than planting geometry of 25 x 15 cm and 25 x 20 cm whereas similar increase in number of siliquae/plant was significant over 30 x 20 cm (Table 1). Plant spacing of 30 x 20 cm registered highest dry matter accumulation and was at par with the 25 x 20 cm plant spacing. Wider spacing increased the branching and resulted in more dry matter accumulation/plant. Planting geometry of 30 x 20 cm registered significantly more LAI than 25 x 15 cm and 25 x 20 cm and number of secondary branches/plant over 25 x 15 cm. Plant spacing of 30 x 20 cm registered significantly higher 1000 seed weight than 25 x 15 cm spacing.

Table 1 Effect of treatments on growth and yield attribute of Indian mustard (pooled data of two years)

Treatments	Plant height at 120 DAS	Dry matter accumulation (g/plant) at 60 DAS	Leaf Area Index at 60 DAS	Number of primary branches/plant	Number of secondary branches/plant	Number of siliqua/plant	Seeds/ siliqua	1000 seed weight (g)	Seed yield (kg/ha)	Stover yield (kg/ha)	Harvest Index (%)
Doses of nutrients (kg N, P_2O_5 and K_2O/ha)											
30:20:20	119.5	33.2	2.4	5.3	7.2	278	7.3	3.1	871	2392	26.69
45:30:30	123.9	34.8	2.4	6.4	8.1	299	7.7	3.1	1183	3493	25.30
60:40:40	133.8	40.5	2.9	7.1	9.3	324	9.8	3.8	1674	4881	25.54
75:50:50	137.2	40.1	3.1	7.9	10.0	434	11.3	4.1	1751	5155	25.35
90:60:60	141.1	39.6	3.1	8.2	9.0	353	10.2	4.1	1733	6081	22.18
SEm±	2.0	0.5	0.2	0.4	0.3	7.6	0.3	0.2	26.2	78.7	0.35
CD (p=0.05)	4.8	1.6	0.5	1.1	0.9	21.0	0.8	0.6	74.4	220.3	0.98
Planting geometry (cm)											
25 x 15	121.2	30.3	2.2	6.2	7.8	279	8.0	3.3	1246	3120	28.54
25 x 20	126.1	42.7	2.4	7.2	9.0	332	9.4	3.8	1644	5113	24.33
30 x 15	141.0	34.1	3.0	7.0	8.9	405	10.0	3.5	1501	4908	23.42
30 x 20	136.0	43.5	3.5	7.5	9.1	335	9.6	4.0	1379	4461	23.61
SEm±	1.8	0.6	0.2	0.3	0.3	7.0	0.2	0.2	20.9	68.0	0.29
CD (p=0.05)	5.5	1.7	0.6	0.9	0.8	23.1	0.7	0.7	61.0	213.6	0.78

Seed yield obtained with the 25 x 20 cm spacing (1644 kg/ha) was significantly higher than all other spacing treatments. Planting geometry of 25 x 20 cm registered 24.2 % more yield over plant spacing of 25 x 15 cm. The dense plant population reduced the yield due to reduction in the photosynthetically active leaf area caused by mutual shading (Mukherjee, 2010). This treatment was followed by 30 x 15 cm and 30 x 20 cm spacing. Similarly plant spacing of 25 x 20 cm registered significantly more stover yield (5113 kg/ha) than 25 x 15 cm and 30 x 15 cm spacing. Plant spacing with 25 x 15 cm spacing registered significantly more harvest index than all other treatments of planting geometry. Planting geometry of 30 x 15 cm resulted in highest N uptake in seed, stover and total uptake though such increases were significant over 25 x 15 cm only in case of seed and total N uptake (Table 2). The P uptake by seed, stover and total registered in case of 25 x 20 cm spacing were significantly higher than 25 x 15 cm spacing. Similarly K uptake by stover

and total registered in case of 25 x 20 cm were significantly higher than 25 x 15 cm and 30 x 20 cm spacing. Sulphur uptake with planting geometry of 30 x 15 cm was significantly higher than planting geometry of 25 x 15 cm. Closer spacing (25 x 20 and 25 x 15 cm) resulted in significantly higher oil content and wider spacing (30 x 20 and 30 x 15 cm) in significantly higher protein content over other crop geometries. Similar result was observed by Yadav *et al.* (2010). Crop geometry of 25 x 20 cm registered highest oil yield which was comparable with that of 30 x 15 cm spacing. However, higher protein yield was obtained with the 30 x 30 cm spacing closely followed by 25 x 20 cm and 30 x 20 cm plant spacing (Table 3). The highest net return (₹43,260) was obtained with the 25 x 20 cm plant spacing with B:C ratio of 3.32. However, maximum benefit: cost ratio (3.51) was recorded with the plant spacing of 30 x 15 cm. Interaction effects of spacing and nutrient doses for various growth and yield attributes and yield were non-significant.

Thus application of 75:50:50 kg N, P_2O_5 and K_2O/ha, respectively resulted in higher seed and oil yields compared to lower doses of nutrients. Crop geometry of 25 cm x 20 cm was found optimum for irrigated timely sown Indian mustard for higher net returns and benefit: cost ratio under alluvial zone of West Bengal.

Table 2 Effect of treatments on nutrient content (%) and uptake by Indian mustard (pooled data of two years)

Treatments	N content (%)		Phosphorus content (%)		Potassium content (%)		Sulphur content (%)		N uptake (kg/ha)			P uptake (kg/ha)			K uptake (kg/ha)			S uptake (kg/ha)		
	Seed	Stover	Seed	Stover	Seed	Stover	Seed	Stover	Seed	Stover	Total	Seed	Stover	Total	Seed	Stover	Total	Seed	Stover	Total
Doses of nutrients (kg N, P_2O_5 and K_2O/ha)																				
30:20:20	1.41	0.54	0.48	0.20	0.63	0.94	0.28	0.53	12.28	12.92	25.20	4.18	4.78	8.96	5.49	22.48	27.97	2.44	12.68	15.12
45:30:30	1.97	0.57	0.51	0.27	0.66	0.94	0.31	0.54	23.31	19.91	43.22	6.03	9.43	15.46	7.81	32.83	40.64	3.67	18.86	22.53
60:40:40	2.26	0. 61	0.53	0.27	0.71	0.99	0.30	0.57	37.83	29.77	67.60	8.87	13.20	22.07	11.89	48.32	60.21	5.02	27.82	32.84
75:50:50	2.34	0.63	0.55	0.28	0.75	0.96	0.31	0.56	40.97	32.48	73.45	9.63	14.40	24.03	13.13	49.49	62.62	5.43	28.87	34.30
90:60:60	2.30	0.62	0.48	0.26	0.75	0.97	0.30	0.58	39.86	37.70	77.56	8.32	15.80	24.12	13.00	58.99	71.98	5.20	35.27	40.47
SEm±	0.17	0.04	0.03	0.04	0.05	0.04	0.03	0.002	0.63	0.46	2.83	0.17	0.24	1.25	0.19	0.83	1.21	0.28	0.58	1.62
CD (p=0.05)	0.50	NS	0.08	NS	NS	NS	NS	0.005	1.81	1.38	6.44	0.50	0.58	3.62	0.55	2.31	3.56	0.64	1.61	4.14
Planting geometry (cm)																				
25x15	1.8	0.49	0.47	0.23	0.61	0.91	0.31	0.50	22.43	15.29	37.72	5.86	7.176	13.03	7.60	28.39	35.99	3.86	15.60	19.46
25 x 20	1.97	0.55	0.49	0.25	0.68	0.97	0.32	0.54	32.39	28.12	60.51	8.06	12.78	20.84	11.18	49.60	60.78	5.26	27.61	32.87
30 x 15	2.19	0.61	0.51	0.26	0.75	0.97	0.29	0.59	32.87	29.94	62.81	7.66	12.76	20.42	11.26	47.61	58.87	4.35	28.96	33.31
30 x 20	2.26	0.67	0.58	0.26	0.77	0.99	0.31	0.58	31.17	29.89	61.05	8.01	11.60	19.61	10.62	44.16	54.78	4.27	25.87	30.15
SEm±	0.16	0.05	0.08	0.06	0.04	0.06	0.04	0.005	0.71	0.55	1.87	0.19	0.28	1.34	0.14	0.86	1.26	0.24	0.62	1.53
CD (p=0.05)	NS	0.14	NS	NS	0.11	NS	NS	0.013	2.30	1.52	5.37	0.55	0.72	3.70	0.41	2.40	3.66	0.69	1.90	4.31

NS = Non significant

Table 3 Effect of treatments on oil, protein and economics of Indian mustard (pooled data of two years)

Treatments	Oil content (%)	Oil yield (kg/ha)	Protein content (%)	Protein yield (kg/ha)	Economics (10^3 x ₹/ha)		
					Gross Return	Net return	Benefit : cost ratio
Doses of nutrients (kg N, P_2O_5 and K_2O/ha)							
30:20:20	38.4	317.4	8.8	76.7	41.98	19.83	1.90
45:30:30	41.5	491.8	12.3	145.6	47.87	30.54	2.76
60:40:40	39.9	668.2	14.1	236.4	60.78	37.61	2.62
75:50:50	38.6	676.7	14.6	256.1	64.81	43.25	3.01
90:60:60	37.9	657.6	14.4	249.1	67.19	41.54	2.62
SEm±	0.4	50.3	0.3	6.2	2.31	1.36	0.32
CD (p=0.05)	1.2	132.2	0.8	15.6	6.45	3.90	0.90
Planting geometry (cm)							
25 x 15	39.7	495.2	11.3	140.1	63.86	27.81	1.77
25 x 20	39.8	655.3	12.3	202.4	61.89	43.26	3.32
30 x 15	38.5	578.4	13.7	205.4	51.58	36.87	3.51
35 x 20	37.5	517.2	14.1	194.7	48.75	30.20	2.63
SEm±	0.4	46.7	0.4	4.1	2.44	1.25	0.28
CD (p=0.05)	1.0	134.5	1.1	11.0	7.54	3.49	0.67

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INDIAN SOCIETY OF OILSEEDS RESEARCH
Instructions to Authors for Preparation of Manuscript for Journal of Oilseeds Research

Prospective author(s) are advised to consult **Issue No. 27(1) June, 2010 of the Journal of Oilseeds Research** and get acquainted with the minor details of the format and style of the Journal. Meticulous compliance with the instructions given below will help quick handling of the manuscript by the reviewers, editor and printers. **Manuscripts are considered for publication in the Journal only from members of the ISOR.**

General

Full-length articles, short communications, book reviews and review articles are published in the Journal. Review articles and book reviews are published usually by invitation. Full length articles and short communications should report results of original investigations in oilseeds, oil bearing plants and relevant fields of science. Choice of submitting the paper(s) either as full length paper or short communication rests with the authors. The Editor(s) or Reviewer(s) will examine their suitability or otherwise only in that specific category. Each article should be written in English correctly, clearly, objectively and concisely. All the statements made in the manuscript should be clear, unambiguous, and to the point. Plagiarism is a crime and therefore, no part of the previously published material can be reproduced exactly without prior permission from the original publisher or author(s) as deemed essential and the responsibility of this solely rests on the authors. Also, authors shall be solely responsible for the authenticity of the results published as well as the inferences drawn thereof. Telegraphic languages should be avoided. The data should be reported in a coherent sequence. Use active voice. Active voice is clear, unambiguous and takes less space. Use past tense while reporting results. Do not repeat ideas in different forms of sentences. Avoid superfluous sentences such as `it is interesting to note that', `it is evident from the table that' or `it may be concluded that' etc. Use % for percent, %age for percentage, / for per, @ for at the rate of hr for hours, sec for seconds. Indicate date as 21 January 2010 (no commas anywhere). Spell out the standard abbreviations when first mentioned eg. Net assimilation rate (NAR), general combining ability (GCA), genetic advance (GA), total bright leaf equivalents (TBLE), mean sum of squares (MSS).

Manuscript

Language of the Journal is English. Generally, the length of an article should not exceed 3,000 words in the case of full-length article and 750 words in the case of short communication. However completeness of information is more important. Each half-page table or illustration should be taken as equivalent to 200 words. It is desirable to submit manuscript in the form of soft copy either as an e-mail attachment to editorisor@gmail.com (preferred because of ease in handling during review process) or in a **compact disk (CD) (in MS Word document; double line space; Times New Roman; font size 12).** In exceptional cases, where the typed manuscript is being submitted as hard copy, typing must be done only on one side of the paper, leaving sufficient margin, at least 4 cm on the left hand side and 3 cm on the other three sides. Faded typewriter ribbon should not be used. Double space typing is essential throughout the manuscript, right from the **Title** through **References** (except tables), foot note etc. Typed manuscript complete in all respects, is to be submitted to the Editor, Journal of Oilseeds Research, Directorate of Oilseeds Research, Rajendranagar, Hyderabad-500 030. Every page of the manuscript, including the title page, references, tables, etc. should be numbered. Punctuation marks help to show the meanings of words by grouping them into sentences, clauses, and phrases and in other ways. These marks should be used in proper manner if the reader of a paper is to understand exactly the intended meaning. Receipt of the manuscript (in the form of either soft or hard copy) will be acknowledged by the editorial office of the Society, giving a manuscript number which should be quoted in all subsequent correspondence regarding that particular article.

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Before reading the instructions given below, the author(s) would better have a close look at the latest issue of the Journal.

Full-length article comprises the following sections.

(a) Short title
(b) Title
(c) Author/Authors
(d) Institution and Address with PIN (postal) code
(e) Abstract (along with key words)
(f) Introduction
(g) Materials and Methods
(h) Results and Discussion
(i) Acknowledgments (if any)
(j) References
(k) Tables and figures (if any)

Guidelines for each section are as follows:

All these headings or matter thereof should start from left hand side of the margin, without any indent.

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A shortened title (approximately of 30 characters) set in capital letters should convey the main theme of the paper.

Title

Except for prepositions, conjunctions, pronouns and articles, the first letter of each word should be in capital letter. The title should be short and should contain key words and phrases to indicate the contents of the paper and be attractive. Jargons and telegraphic words should be avoided. In many cases, actual reading of the paper may depend on the attractiveness of the title.

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The name(s) of author(s) should be typed in capital letters a little below the title, starting from the left margin. Put an asterisk on the name of the corresponding author. **Give the Email ID of the corresponding author** as a footnote.

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This matter will come below the name(s) of the author(s). Name of the Laboratory/Department, followed by the name of the Institution/Organization/University where the work reported in the paper was carried out shall come below the name(s) of author(s). Complete postal address, which should include city/town, district, and state, followed by PIN (postal) code is to be furnished. In case any author has left the above address, this should be indicated as a footnote.

Abstract

The paragraph should start with the word Abstract (in bold font). The abstract should comprise brief and factual summary or salient points of the contents and the conclusions of the investigation reported in the paper and should refer to any new information therein. As the abstract is an independent entity, it should be able to convey the gist of the paper in a concise manner. It will be seen by many more people than will read the paper. The abstract, as concise as possible, should not exceed 250 words in length. Everything that is important in the paper must be reflected in the abstract. It should provide to the reader very briefly the rationale, objectives or hypothesis, methods, results and conclusions of the study described in the paper. In the abstract, do not deflect the reader with promises such as 'will be discussed' or 'will be explained'. Also do not include reference, figure or table citation. At first mention in the abstract, give complete scientific name for plants and other organisms, the full names of chemicals and the description of soil order/series. Any such names or descriptions from the abstract need not be repeated in the text. It must be remembered that the abstracting journals place a great emphasis on the abstract in the selection of papers for abstracting. If properly prepared, they may reproduce it verbatim.

"Key words" should, follow separately after the last sentence of the abstract. "Key words" indicate the most important materials, operations, or ideas covered in the paper. Key words are used in indexing the articles.

Introduction (To be typed as side-heading, starting from the left-hand margin, a few spaces below the key words)

This section is meant to introduce the subject of the paper. Introduction should be short, concise and indicate the objectives and scope of the investigation. To orient readers, give a brief reference to previous concepts and research. Limit literature references to essential information. When new references are available, do not use old references unless it is of historical importance or a landmark in that field. Emphasis should be given among other things on citing the literature on work done under Indian conditions. Introduction must include: (a) a brief statement of the problem, justifying the need for doing the work or the hypothesis on which the work is based, (b) the findings of others that will be further developed or challenged, and (c) an explanation of the approach to be followed and the objectives of the research described in the paper. If the methods employed in the paper are new, it must be indicated in the introduction section.

Materials and methods (To be typed as side-heading, starting from the left-hand margin, a few spaces below the introduction)

This part of the text should comprise the materials used in the investigation, methods of experiment and analysis adopted. This portion should be self-explanatory and have the requisite information needed for understanding and assessing the results reported subsequently. Enough details should be provided in this section to allow a competent scientist to repeat the experiments, mentally or in fact. The geographical position of soil site or soils used in the experiment or site of field trial should be identified clearly with the help of coordinates (latitude & longitude) and invariably proper classification according to Soil Taxonomy (USDA), must be indicated to the level of Great-group, Suborder or Order as far as possible. Specify the period during which the experiment(s) was conducted. Send the article after completion of the experiment(s) not after a gap of 5 years. Instead of kharif and rabi use rainy and winter season respectively. Please give invariably the botanical names for local crop names like raya, bajra moong, cholam etc. Botanical and zoological names should confirm to the international rules. Give authorities. Go through some of our recent issues and find out the correct names. Give latest correct names from authentic source. For materials, give the appropriate technical specifications and quantities and source or method of preparation. Should a product be identified by trade name, add the name and location of the manufacturer or a major distributor in parenthesis after the first mention of the product. For the name of plant protection chemicals, give popular scientific names (first letter small), not trade names (When trade name is given in addition, capitalize the first letter of the name). Known methods of analysis should be indicated by referring to the original source, avoiding detailed description. Any new technique developed and followed should be described in fair detail. When some specially procured or proprietary materials are used, give their pertinent chemical and physical properties. References for the methods used in the study should be cited. If the techniques are widely familiar, use only their names in that case.

Results and Discussion (To be typed as a side-heading, a few spaces below the matter on "Materials and Methods")

This section should discuss the salient points of observation and critical interpretation thereof in past tense. This should not be descriptive and mere recital of the data presented in the tables and diagrams. Unnecessary details must be avoided but at the same time significant findings and special features should be highlighted. For systematic discussion, this section may be divided into sub-sections under side-heading and/or paragraph side heading. Relate the results to your objectives. While discussing the results, give particular attention to the problem, question or hypothesis presented in the introduction. Explain the principles, relationships, and generalizations that can be supported by the results. Point out any exceptions. Explain how the results relate to previous findings, support, contradict or simply add as data. Use the Discussion section to focus on the meaning of your findings rather than recapitulating them. Scientific speculation is encouraged but it should be reasonable and firmly founded in observations. When results differ from previous results, possible explanations should be given. Controversial issues should be discussed clearly. References to published work should be cited in the text by the name(s) of author(s) as follows: Mukherjee and Mitra (1942) have shown or It has been shown (Mukherjee and Mitra, 1942)..... If there are more than two authors, this should be indicated by et al. after the surname of the first author, e.g., Mukherjee et al. (1938).

Always conclude the article by clearly crystallizing the summary of the results obtained along with their implications in solution of the practical problems or contribution to the advancement of the scientific knowledge.

Acknowledgments (To be typed as given above, as a side-heading, well below the concluding portion of Conclusions)

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Tables should not form more than 20% of the text. Each table should be typed on separate sheet and should have on the top a table number (in Arabic numerals *viz*. 1, 2, 3 etc.) and a caption or title which should be short, but sufficiently explanatory of the data included in the table. Information in the table should never duplicate that in the text and vice versa. Symbols (asterisks, daggers, etc. or small letters, *viz*., a, b, etc.) should be used to indicate footnotes to tables. Maximum size of table acceptable is what can be conveniently composed within one full printed page of the journal. Over-sized tables will be rejected out-right. Such tables may be suitably split into two or more small tables.

The data in tables should be corrected to minimum place of decimal so as to make it more meaningful. Do not use full stop with CD, SEm $\pm$, NS (not C.D., S.E.m $\pm$, N.S.). Do not put cross-rules inside the table. Tables should be numbered consecutively and their approximate positions indicated in the margin of the manuscript. Tables should not be inserted in the body of the text. Type each table on a separate sheet. Do not use capital letters for the tabular headings, do not underline the words and do not use a full-stop at the end of the heading. All the tables should be tagged with the main body of the text i.e. after references.

Figures

Figures include diagrams and photographs. Laser print outs of line diagrams are acceptable while dot-matrix print outs will be rejected. Alternatively, each illustration can be drawn on white art card or tracing cloth/ paper, using proper stencil. The lines should be bold and of uniform thickness. The numbers and letterings must be stenciled; free-hand drawing will not be accepted. Size of the illustrations as well as numbers, and letterings should be sufficiently large to stand suitable reduction in size. Overall size of the illustrations should be such that on reduction, the size will be the width of single or double column of the printed page of the Journal. Legends, if any, should be included within the illustration. Each illustration should have a number followed by a caption typed/ typeset well below the illustration.

Title of the article and name(s) of the author(s) should be written sufficiently below the caption. The photographs (black and white) should have a glossy finish with sharp contrast between the light and the dark areas. Colour photographs/ figures are not normally accepted. One set of the original figures must be submitted along with the manuscript, while the second set can be photocopy. The illustrations should be numbered consecutively in the order in which they are mentioned in the text. The position of each figure should be indicated in the margin of the text. The photographs should be securely enclosed with the manuscript after placing them in hard board pouches so that there may not be any crack or fold. Photographs should preferably be 8.5 cm or 17 cm wide or double the size. The captions for all the illustrations (including photographs) should be typed on a separate sheet of paper and placed after the tables.

Expression of Plant Nutrients on Elemental Basis

The amounts and proportions of nutrient elements must be expressed in elemental forms e.g. for ion uptake or in other ways as needed for theoretical purposes. In expressing doses of nitrogen, phosphatic, and potassic fertilizers also these should be in the form of N, P and K, respectively. While these should be expressed in terms of kg/ha for field experiments, for pot culture studies the unit should be in mg/kg soil.

SI Units and Symbols

SI Units (System International d 'Unities or International System of Units) should be used. The SI contains three classes of units: (i) base units, (ii) derived units, and (iii) supplementary units. To denote multiples and sub-multiples of units, standard abbreviations are to be used. Clark's Tables: Science Data Book by Orient Longman, New Delhi (1982) may be consulted.

Some of these units along with the corresponding symbols are reproduced for the sake of convenience.

Names and Symbols of SI Units

Physical Symbol for SI Unit Symbol Remarks quantity physical quantity for SI Unit

Primary Units

length	l	time	t
metre	m	second	s
mass	m	electric current	I
kilogram	kg	ampere	A

Secondary Units

plane angle	radian	rad	Solid angle	steradian	sr

Unit Symbols

centimetre	cm	microgram	µg
cubic centimetre	cm^3	micron	µm
cubic metre	m^3	micronmol	µmol
day	d	milligram	mg
decisiemens	dS	millilitre	mL
degree-Celsium	°C [=(F-32)x0.556]	minute	min

gram	g	nanometre	nm
hectare	ha	newton	N
hour	h	pascal	Pa
joule J	(= 10^7 erg or 4.19 cal.)	second	s
kelvin	K (= °C + 273)	square centimetre	cm^2
kilogram	kg	square kilometre	km^2
kilometre	km	tonne	t
litre	L	watt	W
megagram	Mg		

Some applications along with symbols

adsorption energy	J/mol (=cal/molx4.19)	leaf area	m^2/kg
cation exchange capacity	cmol (p+)/kg (=m.e./100 g)	nutrient content in plants (drymatter basis)	µg/g, mg/g or g/kg
Electrolytic conductivity	dS/m (=mmhos/cm)	root density or root length density	m/m^3
evapotranspiration rate	$m^3/m^2/s$ or m/s	soil bulk density	Mg/m^3 (=g/cm^3)
heat flux	W/m^2	specific heat	J/kg/K
gas diffusion	$g/m^2/s$ or $m^3/m^2/s$ or m/s	specific surface area of soil	m^2/kg
water flow	$kg/m^2/s$ (or) m^3m^2s (or) m/s	thermal conductivity	W/m/K
gas diffusivity	m^2/s	transpiration rate	$mg/m^2/s$
hydraulic conductivity ion uptake	m/s	water content of soil	kg/kg or m^3/m^3
(Per kg of dry plant material)	mol/kg	water tension	kPa (or) MPa

While giving the SI units the first letter should not be in capital i.e cm, not Cm; kg not Kg. There should not be a full stop at the end of the abbreviation: cm, not cm. kg, not kg.; ha, not ha.

In reporting the data, dimensional units, viz., M (mass), L (length), and T (time) should be used as shown under some applications above. Some examples are: 120 kg N/ha; 5 t/ha; 4 dS/m etc.

Special Instructions

I. In a series or range of measurements, mention the unit only at the end, e.g. 2 to 6 cm2, 3, 6, and 9 cm, etc. Similarly use cm2, cm3 instead of sq cm and cu m.

II. Any unfamiliar abbreviation must be identified fully (in parenthesis).

III. A sentence should not begin with an abbreviation.

IV. Numeral should be used whenever it is followed by a unit measure or its abbreviations, e.g., 1 g, 3 m, 5 h, 6 months, etc. Otherwise, words should be used for numbers one to nine and numerals for larger ones except in a series of numbers when numerals should be used for all in the series.

V. Do not abbreviate litre to` l' or tonne to `t'. Instead, spell out.

VI. Before the paper is sent, check carefully all data and text for factual, grammatical and typographical errors.

VII. Do not forget to attach the original signed copy of `Article Certificate' (without any alteration, overwriting or pasting) signed by all authors.

VIII. On revision, please answer all the referees' comments point-wise, indicating the modifications made by you on a separate sheet in duplicate.

IX. If you do not agree with some comments of the referee, modify the article to the extent possible. Give reasons (2 copies on a separate sheet) for your disagreement, with full justification (the article would be examined again).

X. Rupees should be given as per the new symbol approved by Govt. of India.

Details of the peer review process

Manuscripts are received mainly through e-mails and in rare cases, where the authors do not have internet access, hard copies of the manuscripts may be received and processed. Only after the peer review the manuscripts are accepted for publication. So there is no assured publication on submission. The major steps followed during the peer review process are provided below.

Step 1. ***Receipt of manuscript and acknowledgement***: Once the manuscript is received, the contents will be reviewed by the editor/associate editors to assess the scope of the article for publishing in JOR. If found within the scope of the journal, a Manuscript (MS) number is assigned and the same will be intimated to the authors. If the MS is not within the scope and mandate of JOR, then the article will be rejected and the same is communicated to the authors.

Step 2. ***Assigning and sending MS to referees***: Suitable referees will be selected from the panel of experts and the MS (soft copy) will be sent to them for their comments - a standard format of evaluation is provided to the referees for evaluation along with the standard format of the journal articles and the referees will be given 4-5 week time to give their comments. If the comments are not received, reminders will be sent to the referees for expediting the reviewing process and in case there is still no response, the MS will be sent to alternate referees.

Step 3. ***Communication of referee comments to authors for revision***: Once the referee comments and MS (with suggestions/ corrections) are received from the referees, depending on the suggestions, the same will be communicated to the authors with a request to attend to the comments. Authors will be given stipulated time to respond and based on their request, additional time will be given for attending to all the changes as suggested by referees. If the referees suggest no changes and recommend the MS for publication, then the same will be communicated to the authors and the MS will be taken up for editing purpose for publishing. In case the referees suggest that the article cannot be accepted for JOR, then the same will be communicated to the authors with proper rationale and logic as opined by the referees as well as by the editors.

Step 4. ***Sending the revised MS to referees***: Once the authors send the revised version of the articles, depending on the case (like if major revisions were suggested by referees) the corrected MS will be sent to the referees (who had reviewed the article in the first instance) for their comments and further suggestions regarding the acceptability of publication. If only minor revisions had been suggested by referees, then the editors would look into the issues and decide take a call.

Step 5. ***Sending the MS to authors for further revision***: In case referees suggest further modifications, then the same will be communicated to the authors with a request to incorporate the suggested changes. If the referees suggest acceptance of the MS for publication, then the MS will be accepted for publication in the journal and the same will be communicated to the authors. Rarely, at this stage also MS would be rejected if the referees are not satisfied with the modifications and the reasoning provided by the authors.

Step 6. ***Second time revised articles received from authors and decision taken***: In case the second time revised article satisfies all the queries raised by referees, then the MS will be accepted and if not satisfied the article will be rejected. The accepted MS will be taken for editing process where emphasis will be given to the language, content flow and format of the article.

Then the journal issue will be slated for printing and also the pdf version of the journal issue will be hosted on journal webpage.

Important Instructions

- Data on field experiments have to be at least for a period of 2-3 years
- Papers on pot experiments will be considered for publication only as short communications
- Giving coefficient of variation in the case of field experiments Standard error in the case of laboratory determination is mandatory. For rigorous statistical treatment, journals like Journal of Agricultural Science Cambridge, Experimental Agriculture and Soil Use and Management should serve as eye openers.

SPECIAL ANNOUNCEMENT

In a recently conducted Executive Committee meeting of the Indian Society of Oilseeds Research, it was decided to increase the scope of the Journal of Oilseeds Research by accommodating vibrant aspects of scientific communication. It has been felt that, the horizon of scientific reporting could be expanded by including the following types of articles in addition to the Research Articles, Shor Communications and Review Articles that are being published in the journal as of now.

Research accounts (not exceeding 4000 words, with cited references preferably limited to about 40-50 in number): These are the articles that provide an overview of the research work carried out in the author(s)' laboratory, and be based on a body of their published work. The articles must provide appropriate background to the area in a brief introduction so that it could place the author(s)' work in a proper perspective. This could be published from persons who have pursued a research area for a substantial period dotted with publications and thus research account will provide an overall idea of the progress that has been witnessed in the chosen area of research. In this account, author(s) could also narrate the work of others if that had influenced the course of work in authors' lab.

Correspondence (not exceeding 600 words): This includes letters and technical comments that are of general interest to scientists, on the articles or communications published in Journal of Oilseeds Research within the previous four issues. These letters may be reviewed and edited by the editorial committee before publishing.

Technical notes (less than 1500 words and one or two display items): This type of communication may include technical advances such as new methods, protocols or modifications of the existing methods that help in better output or advances in instrumentation.

News (not exceeding 750 words): This type of communication can cover important scientific events or any other news of interest to scientists in general and vegetable oil research in particular.

Meeting reports (less than 1500 words): It can deal with highlights/technical contents of a conference/ symposium/discussion-meeting, etc. conveying to readers the significance of important advances. Reports must

Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance. It could also include the major recommendations or strategic plans worked out.

Research News (not exceeding 2000 words and 3 display items): These should provide a semi-technical account of recently published advances or important findings that could be adopted in vegetable oil research.

Opinion (less than 1200 words): These articles may present views on issues related to science and scientific activity.

Commentary (less than 2000 words): This type of articles are expected to be expository essays on issues related directly or indirectly to research and other stake holders involved in vegetable oil sector.

Book reviews (not exceeding 1500 words): Books that provide a clear in depth knowledge on oilseeds or oil yielding plants, production, processing, marketing, etc. may be reviewed critically and the utility of such books could be highlighted.

Historical commentary/notes (limited to about 3000 words): These articles may inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science. Illustrations and photographs are welcome. Brief items will also be considered.

Education point (limited to about 2000 words): Such articles could highlight the material(s) available in oilseeds to explain different concepts of genetics, plant breeding and modern agriculture practices.

Note that the references and all other formats of reporting shall remain same as it is for the regular articles and as given in Instructions to Authors

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