

COMPARATIVE APTNESS OF PLANT PRODUCTS WITH CHEMICAL-BASED STANDARD GRAIN PROTECTANT AGAINST *CALLOSBRUCHUS CHINENSIS* L. ATTACKING CHICKPEA GRAINS IN STORAGE

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ABSTRACT

Chickpea has to face stern post-harvest losses in storage due to heavy bruchid infestation. Pulse beetle, *Callosobruchus chinensis* L. is the major insect pest attacking its grains in storage. Powder of black pepper fruit, extract of neem seed and oil of castor seed were selected to compare their insecticidal potency with standard grain protectant (coopex dust), polythene enclosure and untreated clean bags. In addition, the effect of plant materials on germination potential and sensory analysis of grains for acceptance was also studied. Plant materials applied both to cloth bag and chickpea grains therein, provided efficient protection to grains in terms of insect number, damaged grains and percent foreign matter that increased gradually and significantly in un-treated bags after every month. However, all the treatments except control had narrow and almost non-significant spectrum of effectiveness. Moreover, polythene enclosure, coopex dust and all the plant materials were significantly promising in their protection against PB up to six months of storage. For most of the plant materials, there was a sudden increase in insect number in May due to emergence F₁ adults and that decreased in June that might be due to very high temperatures and again increased in July and gradually increasing up to August as these months have relatively higher temperature and humidity, which suited its population dynamics and insect number was the maximum. For some plant materials, non-significant fluctuations regarding the number of damaged grains were observed from June to August. It might be due to increasing effectiveness of some plant materials with the passage of time and/or randomization in taking the samples; however, mostly the trend was increasing towards August. The correlation of temperature for these months with the insect number, damaged grains and foreign matter was significantly positive. After 6-months storage, grains from treated and un-treated bags were subjected to germination test and it was observed that bags with higher pest infestation exhibited lower germination percentage and vice versa. The plant material treated samples provided significantly higher germination than the control. The sensory analysis of grain samples from bags was also carried out to assess the intensity of off-flavor in treated and untreated samples. Only the untreated and the coopex dust treated bags provided significantly higher intensity of off-flavor.

Key words: *Callosobruchus chinensis*, stored chickpea grains, mortality, damage and sensory analysis.

Introduction

Chickpea (*Cicer arietinum* L.) ranks 3rd among pulses on global basis and constitutes 88% of the rain-fed cropping system. It alone contributes about 75% of the pulses grown in Pakistan and shares 15% in total pulse production of the world (FAO, 2012). Chickpea production in Pakistan was 484 thousand tons during 2015 as compared to 399 thousand tons in 2014, which is 21% increase (GOP, 2015) and share of the Punjab province is nearly 80% (Hussain *et al.*, 2015). Due to high protein content, it has become an important component of human diet particularly in developing countries. Chemical composition of chickpea includes 45% starch, 25% protein, 6% sugar, 6% crude fiber, 5% fat, 3% ash, 0.19% calcium and other minerals and vitamins are up to 0.01% (Ravi and Harte, 2009).

Chickpea grains in storage face heavy post-harvest losses due to infestations of insect pests particularly of bruchids, resulting in loss of germination capacity thereby becoming unfit for human consumption (Farukh *et al.*, 2011 and Sarwar *et al.*, 2005). Pulse beetle (PB), *Callosobruchus chinensis* causes massive losses to all pulses in storage conditions. It is a major pest of cowpeas, lentils, green gram and black gram (Park *et al.*, 2003) and also damages other stored grains and their products (Sagheer *et al.*, 2013; Tesfu and Eman, 2013). The female lays eggs, which are glued to the seed surface. The larvae bore into the pulse grain

and feed on the entire material. The adults emerge out leaving behind holed grains, which are unsuitable for human consumption (Raja *et al.*, 2001; Ajayi and Lale, 2001).

Use of synthetic pesticides and fumigants is the most common practice to control insect pests in stored grains/products, causing health problems, residual toxicity, environmental pollution and development of resistance against bruchids (Khan *et al.*, 2015). Contrary to these, plant materials are less toxic to mammals, more selective in action, more readily biodegradable and hinder the resistance. Alternative methods including use of ethno-botanicals to manage stored grains insect infestations are being prioritized, which are non-toxic, environment friendly and human health hazard free. A number of medicinal plants and spices have been used as pest control agents (Ani, 2010; Yankanchi and Gonugade, 2009). Researchers claim the successful use of vegetable oils (Kalita *et al.*, 2014), plant extracts (Chiasson *et al.*, 2004; Devanand and Usha Rani, 2008; Yankanchi, 2009), and botanical powders (Patil *et al.*, 2006; Shukla *et al.*, 2007; Gupta and Srivastav, 2008., Kamran *et al.*, 2015). Bindhu *et al.* (2015) revealed that leaf extracts of *Hydrocotyl asiatica*, *Boerhavia diffusa*, *Bacopa monieri* and *Trichosanthes cucumarina* were toxic against PB.

Indigenous plant materials are cheaper and hazard free in comparison with chemical insecticides. The frequency of application or efficacy of the natural products applied to the stored grains, however, has not been thoroughly investigated. Many of the plant products act as repellents, insecticides or chemo-sterilants against insects and can lead to the development of new classes of safer bio-pesticides. Much effort has, therefore, been focused on plant-derived materials for potentially useful products as commercial insect control agents.

The current studies were therefore conducted to evaluate the aptness of plant-based materials in different formulations with the chemical-based standard grain protectant to manage pulse beetle infestation in chickpea grains for their safer and longer storage. In addition, the effect of plant materials on germination potential and sensory analysis of grains for acceptance was also studied.

Materials And Methods

To execute insect bioassays, culture of *Callosobruchus chinensis* was maintained following Shaheen *et al.* (2006) in "Stored Product Entomology Laboratory" of Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan. The Hessian cloth bags of 30x30 cm² sizes, each containing 5 Kg grains of CM-2000 (chickpea cultivar), were treated with different plant materials and stored under laboratory conditions. Before execution of insect bioassays, chickpea grains were made un-infested, followed by Shaheen *et al.* (2006). Twenty five pairs of adult PB were released in each bag on March 1, 2015.

Prior to execute this study, a pilot scale study was conducted in which powders and extracts of leaves of olive (*Olea europea*), tea (*Thea chinensis*), bhang (*Canabis sativa*), elephanta (*Alocassia sp.*), dharek (*Melia azedarach*), seeds of neem (*Azadirachta indica*), datura (*Datura stramonium*), bulbs of garlic (*Allium sativum*), rhizomes of turmeric (*Curcuma longa*) and fruits of cloves (*Syzygium aromaticum*), black pepper (*Piper nigrum*) and red chilies (*Capsicum annum*) were used against PB. In addition, oils of olive (*Olea europea*), neem (*Azadirachta indica*), garlic (*Allium sativum*), cloves (*Syzygium aromaticum*), coconut (*Cocos nucifera*), castor (*Ricinu communis*), datura (*Datura stramonium*), soybean (*Glycine max*), groundnut (*Arachis hypogaea*), sesame (*Sesamum indicum*), turmeric (*Curcuma longa*) and taramera (*Eruca sativa*) were also used against PB. Based on results, powder of black pepper fruit, extract of neem seed and oil of castor seed were proven the best in effectiveness against PB. Consequently, those were selected to compare their insecticidal potency with standard grain protectant (coopex dust), polythene enclosure and untreated clean bags.

Following treatments were applied to bags, each in three replicates:

- i. Black pepper powder applied to bag @ 1000 µg/cm²
- ii. Black pepper powder applied to grains @ 1000 mg/Kg
- iii. Both bag and grains were treated with black pepper powder as in i and ii
- iv. Castor oil applied to bag @ 1000 µl/cm²
- v. Castor oil applied to grains @ 1000 ml/Kg
- vi. Both bag and grains were treated with castor oil as in iv and v
- vii. Neem extract applied to bag @ 1000 µl/cm²
- viii. Neem extract applied to grains @ 1000 ml/Kg
- ix. Both bag and grains were treated with neem extract as in vii and viii
- x. Standard grain protectant (coopex dust 0.5% permethrin w/w @ 1g/Kg) applied to grains only
- xi. Polythene enclosure (0.2 mm thick sheet)
- xii. Control-untreated clean bags

The treated and untreated bags were randomized and stacked on metallic racks in the laboratory up to six months starting from March 1 to August 31, 2015. The temperature in degree centigrade and percent relative humidity of the laboratory were recorded daily for these months by using thermo-hygrometer and their average for every month were calculated. Grain samples of 100 g were drawn monthly from every bag up to six months using a probe sampler and analyzed for number of damaged/holed grains, number of insects (live adults) and foreign matter (grain loss), which was determined by sieving grains and weighing the sieved material. The following studies were also carried out for bag treatments:

Effect of PB infestations on seed germination:

Random samples of grains from each treatment were subjected to germination tests after 6-months storage. From each bag, 20 randomly chosen seeds were placed on 5 rolls of tissue papers in petri-dishes and wetted with distilled water. All the treatments and the control were replicated thrice. The percentage of germinated seeds was noted and compared with the control. The criteria for seed germination were based on both the radical and plumule emergence. The germination experiments were conducted at temperature range of 20-30°C.

Sensory analysis of the grains for assessing the intensity of off-flavors:

The sensory tests of treated and non-treated samples were carried out after 6-months of storage by using a 6-point scale (ISO) for assessing the intensity of off-flavors. The scale used was: 0=absent, 1=very slight, 2=slight, 3=moderate, 4=moderately pronounced, 5=pronounced and 6=very pronounced. Each level of intensity was converted into scores for statistical analysis. To prepare the samples, grains were selected, washed and soaked for 5-hours in water and were pressure-cooked for 15-minutes in water. The cooked samples in triplicate were presented in coded plates chosen at random. At the consumer level, judges were employed using the single stimulation method to detect intensity of off-flavors and acceptability for consumption (Pacheco *et al.* 1995).

Statistical Analysis:

The data recorded were subjected to statistical analysis as Two Factor Completely Randomized Design (CRD) using MSTAT-C program while SPSS 21.0 for Windows program was used for one factor analysis, Duncan's Multiple Range Test (DMRT) was applied to all the means. Moreover, the graphical work was done using Microsoft Excel program.

Results And Discussion

Black pepper powder, neem extract and castor oil were applied in three ways:

- Only applied to bags [B]
- Only applied to grains [G]
- Both bags and grains were treated [B+G]

Effect on insect population:

Figure 1 shows the number of insects alive per sample taken from bags. The number of PB adults in the control was the maximum after every month throughout these studies and were significantly higher compared to all other treatments; where the insect number gradually increased after every month showing 2.00 insects in March, 17.00 in April, 37.33 in May, 54.00 in June, 61.33 in July and 66.00 insects in August. The bags kept in polythene enclosure showed the minimum of 0.33 insects after March and 0.67 and 1.67 insects after July and August, respectively; there were no insects recorded during April, May and June. The bags having coopex dust treated grains showed no insect during March, April and May but 0.67, 1.33 and 2.67 insects were recorded for months of June, July and August, respectively. Both the treatments having polythene enclosure and coopex dust applied to grains were significantly more effective grain protectants as compared with all the plant materials applied in different ways. There was no insect during March in any of the treatments except that of polythene enclosure and the control. Among plant materials, castor oil [B+G] was the most effective, showing 0.67, 3.67, 2.67, 5.00 and 5.67 adults in April, May, June, July and August, respectively. After six months storage, castor oil [B+G] was the most effective exhibiting 5.67 adults followed by castor oil [G] with 6.67 insects, neem [B+G] with 10.67 insects, neem [G] with 13.33 insects, black pepper [B+G] having 15.00 insects, castor [B] with 16.00 insects, black pepper [G] with 19.33 insects, neem [B] having 20.00 insects and black pepper [B], where the maximum insects (27.00) were recorded. For most of the plant materials, there was a sudden increase in insect number in May due to emergence F₁ adults and that decreased in June that might be due to very high temperatures and again increased in July and gradually increasing up to August as these months have relatively higher temperature and humidity, which suited its population dynamics and insect number was the maximum.

Effect on grains in terms of damage:

Results pertaining to damaged grains by beetles, given various treatments of polythene enclosure, coopex dust and different plant materials are presented in Figure 2. Throughout these studies, the number of damaged grains in the control was the maximum after every month and except in March, damaged grains were significantly higher compared to all other treatments; where the number gradually and significantly increased month after month starting from 4.33 damaged grains in March, 21.67 in April, 93.67 in May, 247.33 in June, 333.00 in July and 363.67 grains in August. Bags kept in polythene enclosure and bags having coopex dust treated grains gave full protection against PB up to storage period of six months. Both the treatments having polythene enclosure and coopex dust were statistically similar with all the treatments up to April, except the control. The maximum protection against PB was observed in castor oil [B+G], where 0.00, 0.00, 7.33, 13.67, 11.00 and 11.33 grains were damaged in March, April, May, June, July and August, respectively. After storage of six months, castor oil [B+G] was the most effective against PB showing 11.33 damaged grains followed by castor oil [G], neem [B+G], black pepper [B+G], neem [G], castor [B], black pepper [G], neem [B] and black pepper [B] with 16.00, 17.00, 25.33, 37.67, 42.33, 48.33, 60.33 and 60.67 damaged grains, respectively. For some plant materials, non-significant fluctuations regarding the number of damaged grains were observed from June to August. It might be due to increasing effectiveness of some plant materials with the passage of time and/or randomization in taking the samples; however, mostly the trend was increasing towards August.

Effect on grains in terms of foreign matter:

Figure 3 gives percent foreign matter produced as a result of insect activity in bags treated with different plant materials applied in three ways, polythene enclosure and coopex dust given to grains and the untreated bags. After six months storage, polythene enclosure treatment provided the minimum (0.11%) foreign matter, which was non-significantly followed by coopex dust, castor oil [B+G], castor oil [G], neem [B+G], neem [G], black pepper [B+G], castor oil [B], black pepper [G], neem [B] and black pepper [B], where the percent foreign matter was 0.12%, 0.13%, 0.19%, 0.22%, 0.30%, 0.42%, 0.45%, 0.51%, 0.65% and 0.69%, respectively. All the treatments during six months were statistically similar with one another except those of neem [B] during June and August and black pepper [B] and the control during May, June, July and August. The maximum foreign matter (3.35%) was observed in the control during August, which was just statistically similar with its value during July but significantly different with all other treatments throughout six months and its values during March, April, May and June. The maximum protection was observed in polythene enclosed bags where the percent foreign matter was 0.01%, 0.02%, 0.04%, 0.09%, 0.11% and 0.11% during March, April, May, June and August, respectively.

In above studies, plant materials applied to both bag and grains inside, provided efficient protection to grains in terms of insect number, damaged grains and percent foreign matter, which increased gradually and significantly in un-treated bags month after month. However, all the treatments except control had narrow and almost non-significant spectrum of effectiveness. Moreover, polythene enclosure, coopex dust and all the plant materials were significantly promising in their protection against PB up to six months of storage.

It can be seen in Table 1 that the correlation of temperature for six months with insect number, damaged grains and foreign matter was significantly positive while with relative humidity, it was positive but non-significant.

Figure 1 Mean number of live adults of PB at given month in stored chickpea bags given various treatments

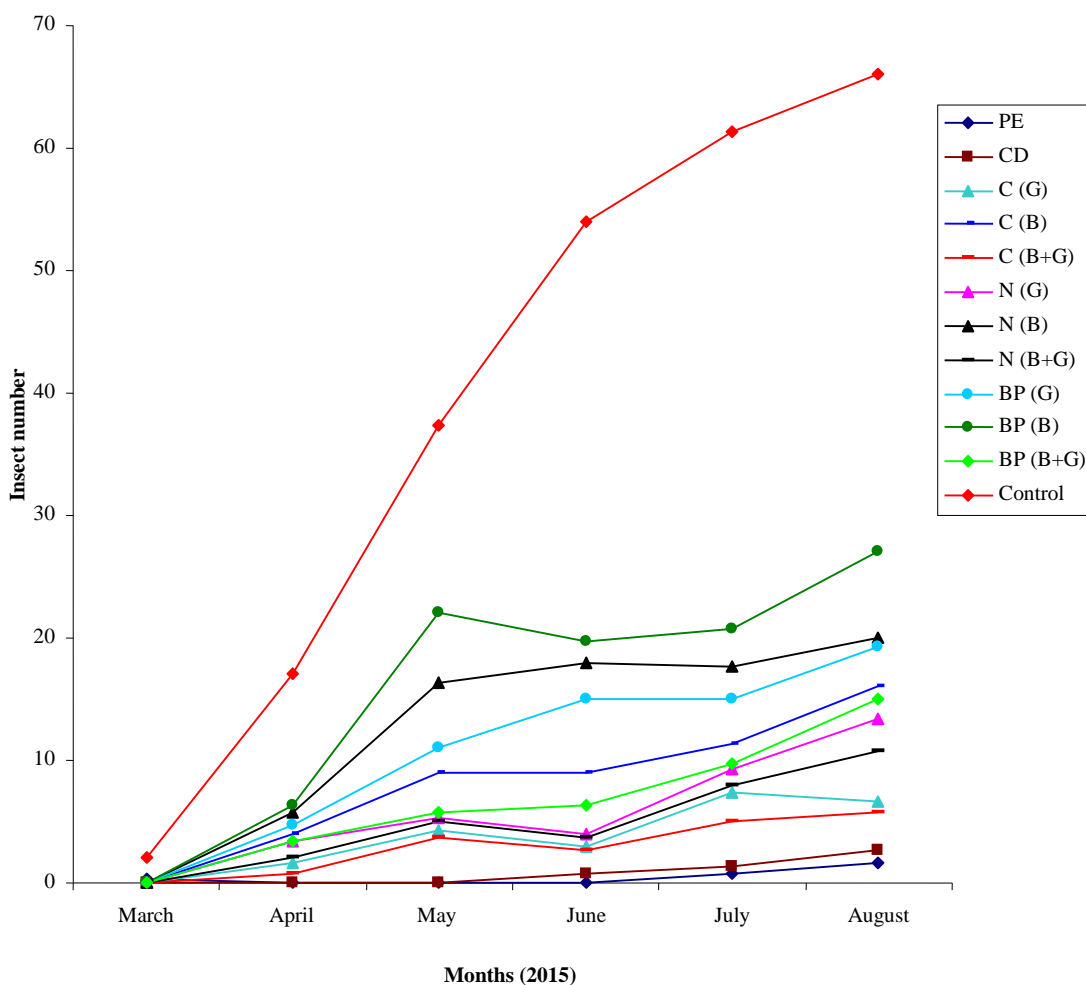
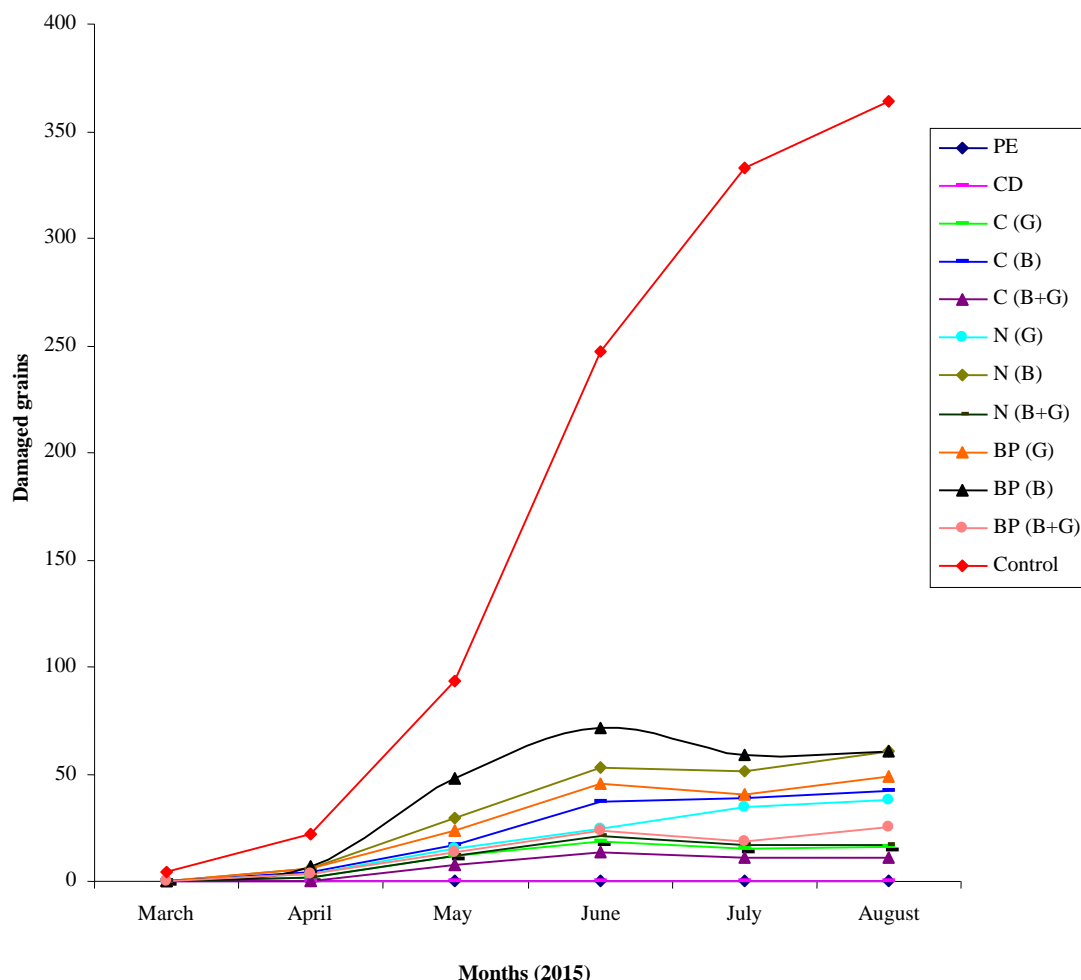


Figure 2 Mean number of damaged grains at given month in stored chickpea bags given various treatments



Effect of PB infestations on seed germination:

The grains from treated and un-treated bags were subjected to germination test after 6-months storage and it was found that polythene enclosure, coopex dust and all the plant materials provided significantly higher germination compared to the control where only 20% seeds germinated (Figure 4). The maximum seed germination (93.33%) was recorded for grains from polythene enclosed bags, which was significantly followed by castor oil [B+G] with 86.67% germination, coopex dust treated grains with 86.67% germination and neem extract [B+G] with 83.33% germination; however, these were non-significant with one another. The percent germination shown by castor oil [G] with 81.67% germination and neem extract [G] with 80.00% germination were statistically similar with neem extract [B+G] but significantly lower compared to polythene enclosed bags, castor oil [B+G] and coopex dust treated grains. All other plant materials with their different applications showed the percent germination ranged from 78.33% to 66.67%.

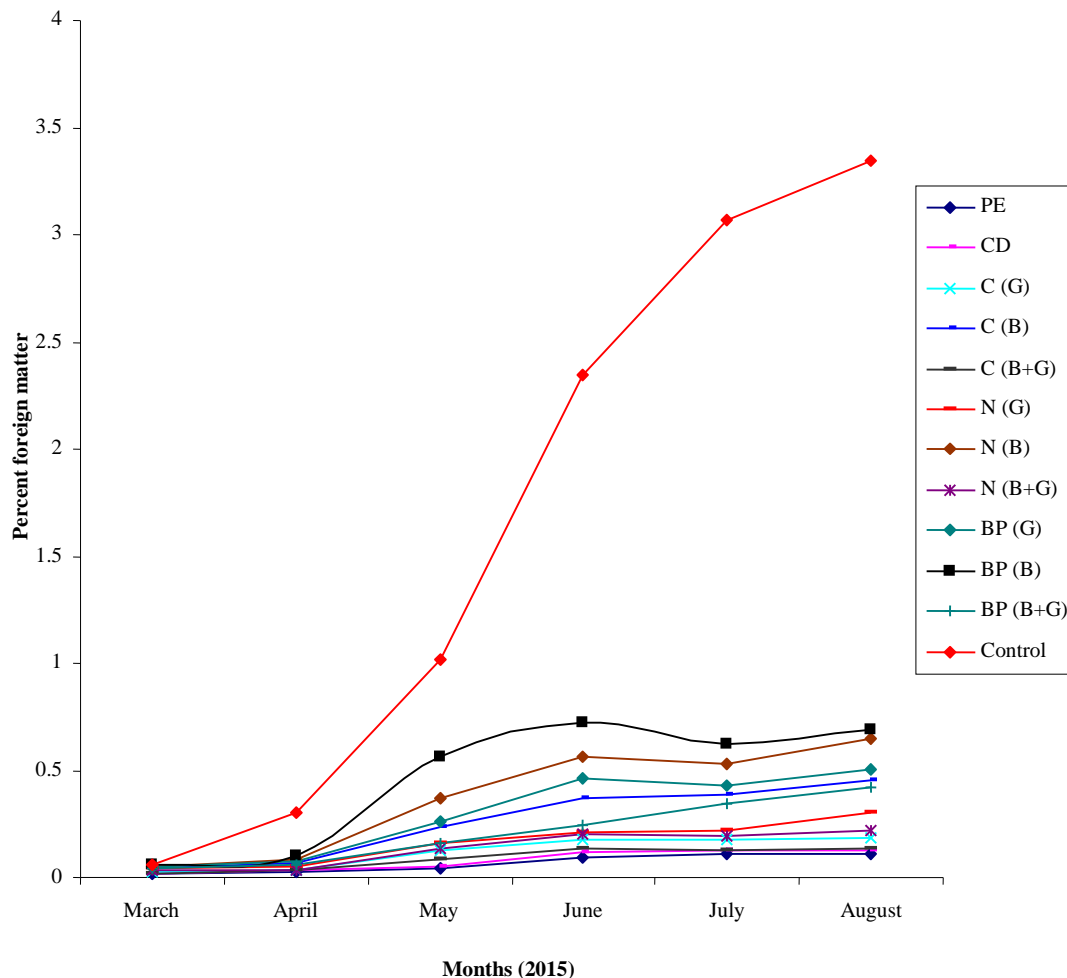
Sensory analysis of the grains for assessing the intensity of off-flavors:

The effect of different bag treatments on intensity of off-flavor in the grains can be seen in Figure 5. The minimum intensity of off-flavor with score of 0.25 was calculated for sample from polythene enclosed bags, which was statistically similar to samples from black pepper powder [B+G] and [G] having scores of 0.67 and 1.08, respectively. Though, the coopex dust was promising in protection of grains against PB up to storage of 6-months but it provided the maximum intensity of off-flavor with score of 5.50 and was non-significantly followed by the control with 4.83 score. All the plant materials with their different applications and the polythene enclosure bags produced significantly lower intensity of off-flavor compared to the control and coopex dust. The intensity of off-flavor caused by plant materials in terms of scores ranged from 0.67 to 3.41.

Figure 6 presents the effect of bag treatments on acceptance for consumption and detection of off-flavor. The results of the consumer acceptance test by single stimulation method showed that 100% of the judges accepted the samples taken from polythene enclosed bags followed by black pepper powder [B+G], black pepper powder [G], neem extract [G], black pepper powder [B], neem extract [B], castor oil [B], neem extract [B+G] and castor oil [G], where the percentages were 91.67, 83.33, 66.67, 66.67, 58.33, 58.33, 50.00 and 50.00. Coopex dust treated grains showed the minimum acceptance of 8.33%, which was followed by control and castor oil [B+G], where the acceptance was 25% and 16.67%, respectively. The minimum detection of off-flavor (16.67%) was calculated for samples taken from the polythene enclosed bags, which was followed by black pepper

powder [B+G], black pepper powder [G], neem extract [G] and black pepper powder [B], where 33.33%, 58.33%, 83.33% and 83.33% off-flavor were detected, respectively. The samples from all other treatments detected 100% off-flavors.

Figure 3 Mean percent foreign matter at given month in stored chickpea bags given various treatments

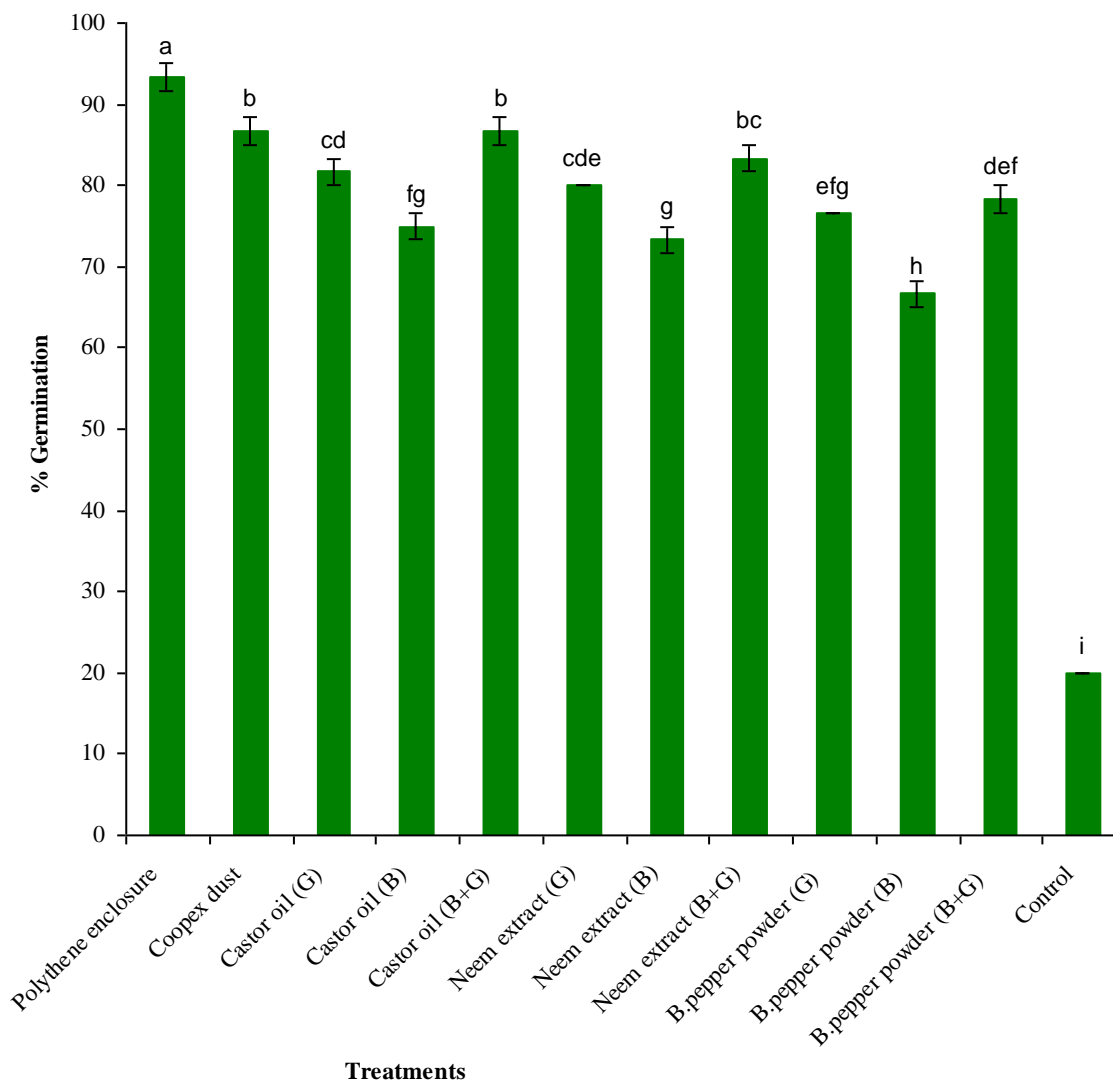


The findings of this research are in conformity with those of Tabu *et al.* (2012), they conducted a study regarding effectiveness of certain botanicals, inert materials and edible oils against *C. chinensis* in stored chickpea. They used four botanicals including *Milletia ferruginea* (seed powder), *Azadrachta indica* (seed powder), *Datura stramonium* (seed and leaf powder), *Chenopodium ambrosioides* (leaf powder), three edible seed oils viz., *Brassica juncea*, *Linum usitatissimum*, *Guizotia abyssinica* and two inert materials of wood ash and sand against this pest. Malathion 5% dust and the control were included for comparison. Plant materials, inert materials and edible seed oils caused high adult mortality, reduced egg laid, reduced F₁ progeny emergence, low seed damage and low seed weight loss and did not affect seed germination in stored chickpea grains. Seed powder of *A. indica* applied at 20 g kg⁻¹ and the leaf powder of *C. ambrosioides* at the rate of 40 g kg⁻¹ caused high adult mortality next to Malathion 5% dust applied @ 0.5g kg⁻¹, whereas the other botanicals, inert materials and oils showed better performance than the untreated check. *B. juncea*, *L. usitatissimum* and *G. abyssinica* seed oils applied @ 5.0 ml kg⁻¹ showed high reduction in progeny emergence. Similarly, sand and wood ash at all the levels tested, gave effective inhibition in F₁ progeny production. In another study, The efficacy of four indigenous plants parts such as leaves of raintree (*Albizia saman*), riot lata (*Mikania micrantha*), pithraj (*Aphanamixis polystachya*) and seeds of mahogani (*Swietenia macrophylla*) with methanol extracts at the different rates were tested against the pulse beetle. Among the four plant extracts tested, pithraj leaf showed maximum repellency effect against the beetle (Ahmed *et al.*, 2014).

Hossain *et al.* (2014) assessed the aptness of 17 indigenous plant powders as grain protectant to control *C. chinensis*. Tobacco leaf powder (TLP) showed promising effects in inhibition of oviposition and reducing adult emergence, seed infestation and grain weight loss by *C. chinensis*. TLP showed full protection of chickpea seeds when applied at 20 g/kg seeds. The lowest number of eggs (24.60), egg bearing seeds (23.40), adult emergence (23.20), seed infestation (8.28%) and weight loss (0.50%) were obtained from TLP treated at 10 g/kg seeds as compared to the highest values in the untreated control. TLP exhibited 100% inhibition of emerged grubs at 20 g/kg seeds and had no adverse effect on seed germination up to 3 months. Gautam *et al.* (2000) used nine edible plant products including black pepper and observed that maximum adult mortality of PB was recorded in cloves followed by black pepper and cinnamon after storage period of 5 months. Studies by Tripathy *et al.* (2001) showed that all the plant-oil treatments were superior in protecting the seeds from PB attack than malathion treatment (60 p.p.m.) or control. The

oils of neem, castor and coconut proved most effective in protecting the seeds for about 9- months after treatment. The powder and extract of *Lantana camara* was promising in protecting the seeds. The use of such botanical products did not affect the permeability of the seeds. Bhuiyah *et al.* (2002) investigated the effect of botanicals for the control of PB attacking lentil and chickpea seeds in storage. The oils of neem, royna and castor whereas leaf powders and crude extracts of neem, bankalmi, bishkatali, marigold, castor and mango were effective in preventing egg-laying of PB. The infestation and weight loss in lentil and chickpea grains were prevented by 100% through treatments with oils, leaf powders and crude extracts of the tested botanicals up to 9-months of storage.

Figure 4 Effect of different bag treatments on percent germination of chickpea grains after their storage of 6-months



Thirteen botanicals were evaluated by Tebkew and Mekasha (2002) for their efficacy to control PB in stored chickpea using the improved variety Marye. Among these botanicals, only two plant species, *Millettia ferruginea* and *Nicotiana tabacum*, gave complete protection for 6-months and had no negative effect on the germination of seeds. Singh (2003) checked the effect of some plant oils including castor oil and found that all the oils were highly effective in protecting the seed up to 9-months storage in terms of seed damage and weight loss. The oils prevented egg-laying and controlled the population build-up of the beetle. Pacheco *et al.* (1995) evaluated refined soybean and crude castor oils for the control of infestations by *C. maculatus* and *C. phaseoli* in stored chickpea. After 5-months of storage, the effect of treatments on consumer acceptability and seed germination were also examined. Both oils inhibited the population growth of these beetles as compared to the untreated seeds; however, castor oil was more effective than soybean oil. No harmful effect was observed on the germination of oil treated seeds. The oil treatments conferred off-flavors to chickpeas and product acceptability was compromised by treatment with castor oil and soybean oil at 10 ml/kg. According to Mendki *et al.* (2001), no adult PB was found in pulses treated with fly-ash even after 12 months of treatment. After 18 months of storage, chickpea was the most infested both in terms of number of insects observed in gunny bags and percent damaged grains. Percentage insect-damaged grains were directly proportional to the number of insects observed in gunny bags. There was no effect of fly-ash on the nutritional quality and percent germination of pulses.

In a similar study by Tesfu and Eman (2013), the aptness of *Parthenium hysterophorus* against *C. chinensis* was tested in the laboratory under ambient condition. Powders of its different plant parts applied to chickpea seeds were toxic to *C. chinensis* and caused significant ($P < 0.05$) mortality after 24 hours except stem powder. The highest dose (2/50 g seed) of inflorescence, leaf and stem powder caused 76.67, 73.33 and 56.67% mortality, respectively. All the powders significantly ($P < 0.05$) induced the emergence of F_1 progeny compared with the untreated chickpea seeds. The powder has also played a significant role in reducing grain weight loss. In an experiment carried out by Zia *et al.* (2011), ten extracts of different plants were tested for their insecticidal potency against *C. chinensis* in chickpea grains. Black pepper (*Piper nigrum*) was found as the most effective in controlling this beetle followed by cloves (*Syzygium aromaticum*), neem (*A. indica*) and garlic (*Allium sativum*).

Figure 5 Effect of different bag treatments on intensity of off-flavor in chickpea grains after their storage of 6-months

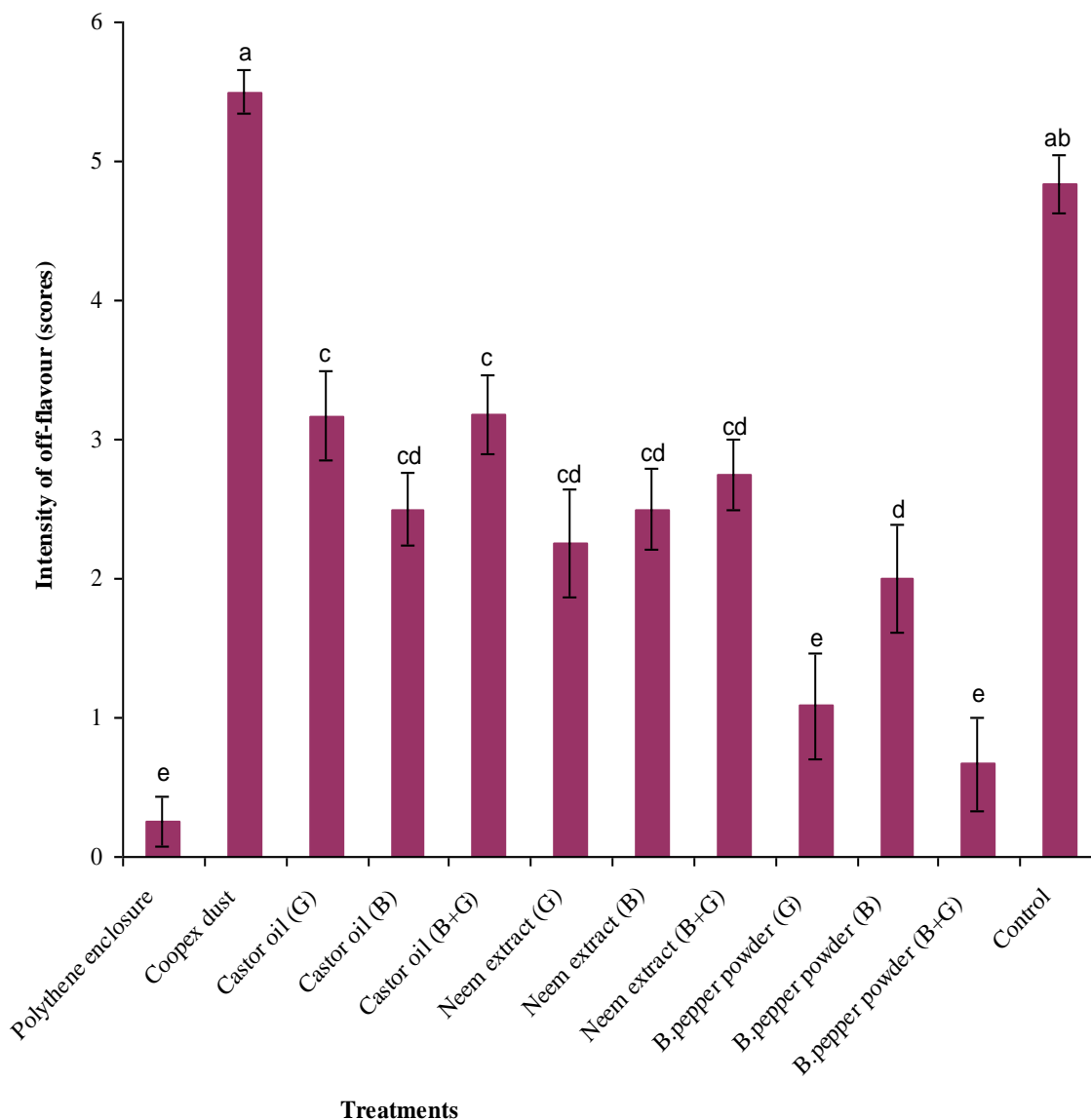


Figure 6 Effect of different bag treatments on acceptance for consumption and detection of off-flavor in grains after their storage of 6-months

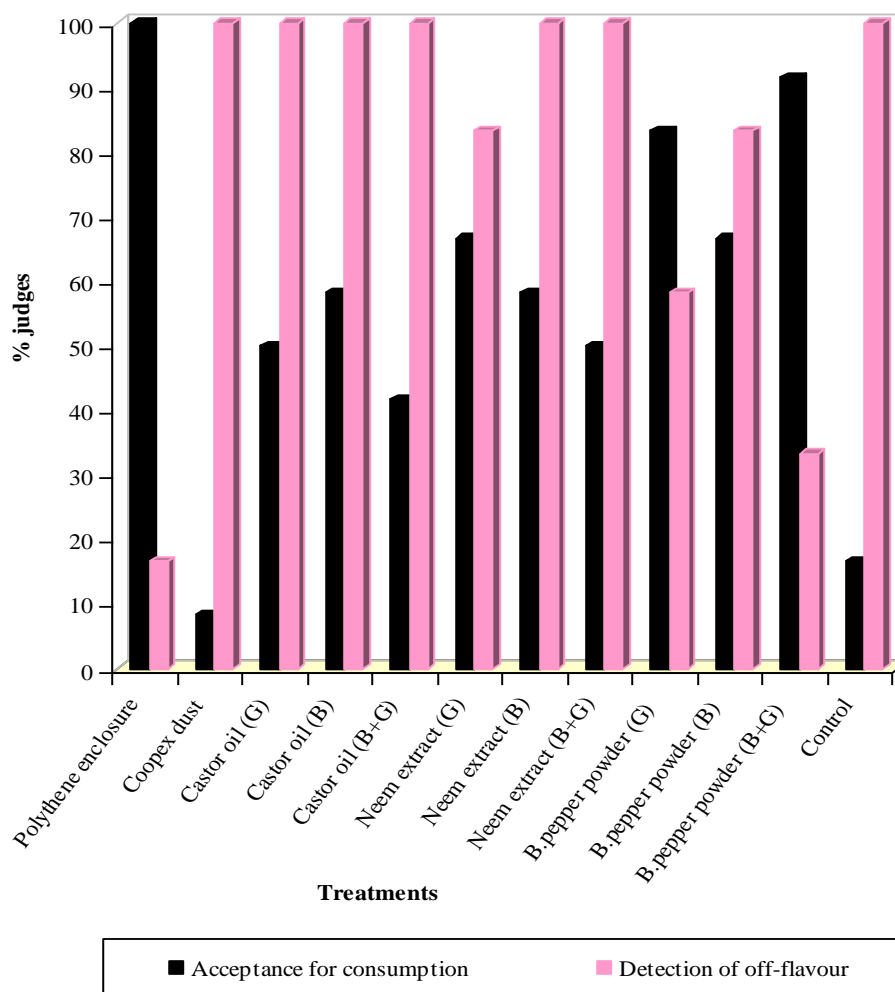


Table 1 Correlation between month, temperature, relative humidity, insect number, damaged grains and foreign matter for bag treatment studies

Pearson Correlation	Month	Temperature	Relative humidity	Insect number	Damaged grains	Foreign matter
Month	1	.783	.551	.981**	.971**	.976**
Temperature	.783	1	-.056	.855*	.840*	.836*
Relative humidity	.551	-.056	1	.409	.485	.491
Insect number	.981**	.855*	.409	1	.957**	.966**
Damaged grains	.971**	.840*	.485	.957**	1	.999**
Foreign matter	.976**	.836*	.491	.966**	.999**	1

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Recommendations

The use of synthetic insecticides as grain protectants or fumigants in Pakistan has created a multitude of problems of toxic residues in food grains and other related health risks. As a huge number of stored product insect pests have developed resistance to these insecticides, there is need to develop alternate management strategies, especially those utilizing renewable resources and/or traditional experiences. Major part of food grains is stored in bags and only a minor part is kept as bulk in the public

sector in Pakistan. Fumigation with aluminium phosphide is the major pest control practice in grain storages where re-infestation, especially at farm level storage, is a continuous threat along with development of resistance in insects against these insecticides. This situation supports the application of alternative measures especially use of plant materials for preventing insect losses to stored grains. Being non-toxic and non-hazardous, these are suited for the management of storage pests.

Farmers should use indigenous plant materials, possessing insecticidal properties, for the management of PB due to their easy and local availability, economic and safe use. The plant powders and extracts of black pepper, red chilies, cloves, neem, datura, garlic and turmeric were found promising against PB but their extract formulations were more effective than their formulations as powder. Plant oils of castor, neem, taramera, cloves, datura, garlic and turmeric showed significant insecticidal potency against PB. Castor and neem oils in equal ratios may be used for their synergistic effect against PB for promising results. However, black pepper as powder, neem as extract and castor as oil appeared to be the most effective formulations and can be efficiently used in managing populations of PB to lower levels.

For storage in bags up to six months, black pepper powder, neem extract and castor oil gave promising protection of chickpea grains against PB. Use of these plant materials also had no effect on the vigour and flavour of the grains. Hence, these studies provide basis for a pest control technology at farm level storage, which can be effectively used in rural areas. Moreover, problem of re-infestation after fumigation in long-term storage may be overcome by integration of promising locally available plant materials with other feasible control measures. This technology will be practicable, economical and preferable by resource limited farmers in developing countries like Pakistan, where air-tight storage structures are beyond their reach.

There is a hectic and needed activity around the globe to develop and commercialize plant based products for insect control. There is also a vast scope in small scale manufacturing of pest control materials. These studies provide a gateway for the researchers/bio-chemists to find out active materials/fractions of these plants for developing economical commercial formulations, which will definitely lead to the development and establishment of bio-pesticides industry in Pakistan and throughout the world. Use of these plant materials in storage can't replace pesticides; however, it can definitely reduce their amount and thus will minimize toxic residues in food grains. The application of these plant materials to stored chickpea in combination with fumigation can be used for integrated pest management of PB, where its infestations are above economic threshold levels.

References

- Ahmed, M. T., Begum, M. and Zaman, M. W. (2014). The repellent effect of some indigenous plant extracts against pulse beetle (*Callosobruchus chinensis*). J. Environ. Sci. & Natural Resources, 7: 151-154.
- Ajayi, F. A. and Lale, N. E. S. (2001). Seed coat texture, host species and time of application affect the efficacy of essential oils applied for the control of *C. maculatus* (F.) (Coleoptera : Bruchidae) in stored pulses. Inter. J. Pest Manag., 43: 161-166.
- Ani, D. S. (2010). Screening of some bio-pesticides for the control of *Callosobruchus chinensis* in stored blackbeans (*Vigna mungo*) in Imo state. J. American Sci. 6: 186-188.
- Bindhu, V., Ganga, S. and Susha, D. (2015). Mortality Effects of Some Medicinal Plants on the Pulse Beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). J. Biofertil Biopestici., 6:1-4
- Bhuiyah, M. I. M., Karim, A. N. M. R., Islam, B. N. and Alam, Z. (2002). Test of botanicals for the control of the beetle attacking lentil and chickpea in storage. Bangladesh J. Agri. Res., 27: 349 – 362.
- Chiasson, H., Vincent, C. and Bostanian, N. J. (2004). Insecticidal properties of a *Chenopodium* based botanical. J. Econ. Entomol. 97: 1378-1383.
- Devanand, P. and Rani, P. U. (2008). Biological potency of certain plant extracts in management of two lepidopteran pests of *Ricinus communis* L., J. Biopesticides, 1: 170-176.
- FAO. (2012). Year Book. Food and Agriculture Organization of the United Nations.
- Faruk K., Varol, I. and Bayram, M. (2011). The effect of carbon dioxide at high pressure under different developmental stages of *Callosobruchus maculatus* (F) hosting on chickpea. Afr. J. Biotechnol. 10(11): 2053-2057.
- GOP. (2015). Pakistan Economic Survey 2014-15. Economic advisor's Wing. Finance Division, Islamabad.
- Gautam, P., Vaidya, D. N. and Mehta, P. K. (2000). Evaluation of some edible plant products against pulse beetle, *Callosobruchus analis* (Fabr.) infesting green gram. Pest Management And Economic Zool. 8: 145-150.
- Gupta, L. and Srivastava, M. (2008). Effect of *Withania somnifera* extracts on the mortality of *Callosobruchus chinensis* L. J. Biopesticides, 1: 190-192.
- Hossain, M. A., Bachchu, M. A. A., Ahmed, K. S. and Haque, M. A. (2014). Effectiveness of indigenous plant powders as grain protectant against *Callosobruchus chinensis* (L.) in stored chickpea (*Cicer arietinum*). Bangladesh J. Agril. Res., 39(1): 93-103.
- Hussain, N., Aslam, M., Ghaffar, A. and Irshad, M. (2015). Chickpea genotypes evaluation for morpho-yield traits under water stress conditions. J. Anim. Plant Sci. 25: 206-211.
- Kamran, A., Adnan M., Khan, M. A., Hussain, Z., Junaid, K., Saleem, N., Ali, M., Basir, A. and Ali, A. (2015). Bioactive neem leaf powder enhances the shelf life of stored mungbean grains and extends protection from pulse beetle. Pak. J. Weed Sci. Res., 21: 71-81.
- Khan, M. Z., Ali, M. R., Bhuiyan, M. S. I. and Hossain, M. A. (2015). Eco-Friendly Management of Pulse Beetle, *Callosobruchus Chinensis* Linn. Using Fumigants on Stored Mungbean. Inter. J. Sci. Res., 5:1-6.

- Kalita, J., Dutta, P., Gogoi, P., Bhattacharyya, P. R. and Nath, S. (2014). Biological Activity of Essential Oils of Two Variant of *Cinnamomum Verum* Presl. From North East India on *Callosobruchus chinensis* (L.). *Intr. J. A. Bio. Phra. Tech.* 5:190-194.
- Mendki, P. S., Maheshwari, V. L., Kothari, R. M. (2001). Flyash as a post-harvest preservative for five commonly utilized pulses. *Crop Protection*, 20: 241-245.
- Pacheco, I. A., Castro, M. F. P. P. M. D., Paula, D. C. D., Lourencao, A. L., Bolonhezi, S. and Barbieri, M. K. (1995). Efficacy of soybean and castor oils in the control of *C. maculatus* and *C. phaseoli* in stored chickpeas. *J. Stored Prod. Res.*, 31 (3): 221-228.
- Park, C., Kim, S. I. and Ahn, Y. J. (2003). Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three Coleopteran stored product insects. *J. S. P. Rese.*, 39: 332-342.
- Patil, P. B., Holihosur, S. N. and Kallapur, V. L. (2006). Efficacy of natural product *Clerodendron inerme* against mosquito vector *Aedes aegypti*. *Current Science*, 90: 1064-1066.
- Raja, N., Alberta, S., Ignacimuthua, S and Dorn, S. (2001). Effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* L. against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) infestation. *J. S. P. Research.* 37: 127-132.
- Ravi, R. and Harte, J.B. (2009). Milling and physicochemical properties of chickpea (*Cicer arietinum* L.) varieties. *J. Sci. Food Agric.* 89: 258-266.
- Sagheer, M., Hassan, M., Ali, Z., Yasir, M., Ali, Q. and Khan, F. Z. A. (2013). Evaluation of essential oils of different citrus species against *Trogoderma granarium* (Coleoptera: Dermestidae) collected from Vehari and Faisalabad districts of Punjab, Pakistan. *Pak. Ento.*, 35: 37-41.
- Sarwar, M., Ahmad, N., Sattar, M. and Tofique, M. (2005). Evaluating the seed's reaction of certain chickpea genotypes against the action of pulse beetle, *Callosobruchus analis* L. (bruchidae: coleoptera). *Pak. J. Seed Technol.* 1: 14-21.
- Shaheen, F.A., Khaliq, A. and Aslam, M. (2006). Resistance of chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. *Pak. J. Bot.* 38: 1237-1244.
- Shukla, R., Srivastava, B., Kumar, R. and Dubey, N. K. (2007). Potential of some botanical powders in reducing infestation of chickpea by *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). *J. Agric. Technology* 3: 11-19.
- Singh, P. K. (2003). Effect of some oils against pulse beetle, *Callosobruchus chinensis* in infesting pigeon pea. *Ind. J. Entomol.*, 65: 55 – 58.
- Tebkew, D. and Mekasha, C. (2002). The efficacy of some botanicals in controlling Adzuki bean beetle, *Callosobruchus chinensis* in stored chickpea. *Tropical-Science*, 42 (4): 192-195.
- Tabu, D., Selvaraj, T., Singh, S. K. and Mulugeta, N. (2012). Management of Adzuki bean beetle (*Callosobruchus chinensis* L.) using some botanicals, inert materials and edible oils in stored chickpea. *Journal of Agricultural Technology*, 8(3): 881-902.
- Tesfu, F. and Emanu, G. (2013). Evaluation of *Parthenium hysterophorus* L. powder against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) on chickpea under laboratory conditions. *African Journal of Agricultural Research*, 8(44): 5405-5410.
- Tripathy, M. K., Sahoo, P., Das, B. C. and Mohanty, S. (2001). Efficacy of botanical oils, plant powders and extracts against *Callosobruchus chinensis* Linn. attacking blackgram (Cv. T9). *Legume Res.*, 24(2): 82-86.
- Yankanchi, S. R. (2009). Efficacy of different solvents extract of *Clerodendrum inerme* Gaertn against larvae of castor semilooper, *Achaea janata* L. *Uttar Pradesh Journal of Zoology*, 29: 299-303.
- Yankanchi, S. R. and Gonugade, R. S. (2009). Antifeedant and insecticidal activities of certain plant extract against Red flour beetle, *Tribolium castaneum* H. J. *Life Science Bulletin.* 6: 331-335.
- Zia, A., Aslam, M., Naz, F. and Illyas, M. (2011). Bio-efficacy of some plant extracts against chickpea beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) attacking chickpea. *Pakistan J. Zool.*, 43 (4): 733-737.