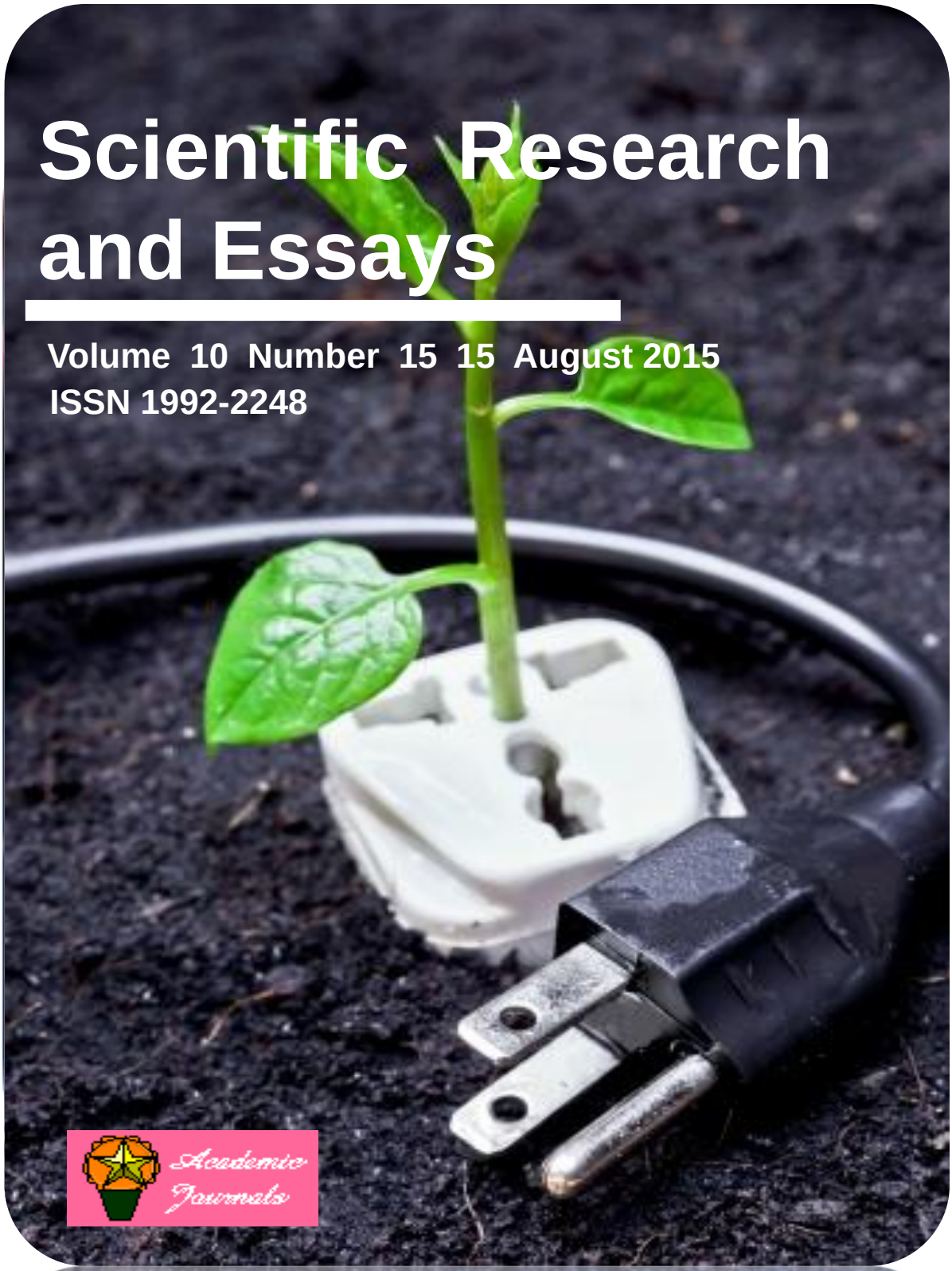

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Full Length Research Paper

Evidence for the presence of a female produced sex pheromone in the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae)

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Behaviour-modifying chemicals like pheromones and kairomones hold a great potential in pest management. Evidences from mating behaviour studies of the banana weevil, and from the weevil's responses to their freeze-killed conspecifics, body washes/extracts, live conspecifics (olfactometer studies), and trapped volatiles of mature and immature adults clearly suggest that two types of pheromones are produced in this insect: a female produced sex pheromone and a male produced aggregation pheromone. Both are perceived by olfactory means. The latter has already been isolated by earlier workers and is in use in control programs. Greater successes may however, be recorded with the control of this pest (e.g. in mating disruptions, mass trappings, pest monitoring) if the female-sex pheromone also gets finally isolated, and used in conjunction with good cultural practices.

Key words: Cosmopolites sordidus, sex pheromone, mating disruptions, bioassays, olfactometer studies, gas chromatographic profiles, electroantennogram studies.

INTRODUCTION

The banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) is recognized as the major insect pest of bananas and plantains (*Musa* spp.) (Ostmark, 1974; Gold et al., 2001). It is known to attack these crops whenever they are cultivated (Zimmerman, 1968; Ostmark, 1974; Pavis, 1988). All four stages of the weevil's life history are associated with the banana plant (Treverrow, 2003). The eggs are usually laid singly and superficially by adult females at the base of the plant, or corm and also in the crop residues Koppenhofer (1993); and upon hatching the larvae which constitutes the most destructive stage of the pest (Jones, 1986) burrows into the stems, weakens them and makes them liable to wind damage (Acland, 1971). Damage to young suckers by a single borer (weevil larva) is almost catastrophic, as the larva eats up a large part of the corm and growing points, setting up secondary rots from which the plant has no chance of recovery (Simmonds and Simmonds, 1953).

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Attacks to older plants however, reduce the vitality and resistance of the plants to drought, leading to the development of poor fruit bunches and stems too weak to resist high winds (Harris, 1947). The fully fed larva normally tunnels to near corm surface to form an oval chamber in which it pupates, just prior to adult emergence (Treverrow, 2003). Emerging adults are nocturnally active, free-living and long-lived (range: 2 - 4 years; Gold et al., 2001).

All species of the genus Musa are attacked by the pest and no banana cultivar (Wolcott, 1933; Simmonds, 1966) is known to have a total resistance to it. Certain cultivars however, are known to be more susceptible to the borers than others (Wolcott, 1933; Viswanath, 1981; Mesquita et al., 1984; Gold et al 2001).

Chemical control with insecticides has been widely used on large commercial plantations and in some areas by small farmers. Prohibitive costs, the harmful effects of insecticides to environment and human health; and the development of resistance by the pest following the use of chemicals (Jones, 1986; Neuenschwander, 1988) have made continual use of chemicals for control of this pest unacceptable. Novel means of control, such as the use of pheromones, in conjunction with good cultural practices currently in use, would help to greatly reduce the losses caused by this pest. Tinzaara et al. (2002) reported that pheromones and other behaviour modifying chemicals (e.g. kairomones) hold a great potential as tools for pest management; particularly for use in pest monitoring, mating disruptions, mass trappings, and even as means for aggregating pests to delivery sites for biological control agents ("lure and kill"). The authors therefore called for further exploits in the synergism between banana plant extracts (kairomones) and the synthetic pheromones in attracting the banana weevil, *C. sordidus*. This study is therefore aimed at investigating this pest for clues about its behaviour-modifying chemicals (specifically for pheromonal involvement in its mating and gregarious behaviour). Such findings (e.g. presence of a sex pheromone) if confirmed, may be exploited for a more effective, novel and integrated control for the pest.

MATERIALS AND METHODS

All bioassays in this study were conducted in a small fan-ventilated room (approximately 2.5 m^2) with controlled temperature and relative humidity conditions (25 ± 2°C and 80 ± 5% respectively). The fluorescent light source in the dark room was covered with a deep red light filter (Kodak Wratten No. 70, transmitting only wavelengths greater than 640 nm, adapted from Budenberg et al. (1993a, b). The dim light made observations in the dark room possible, with no obvious disturbance to responders.

Fresh field-collected weevils that were less than 3 days old in the laboratory were used in all these experiments (bioassays) as either dummies or responders, since they had been found to be more responsive than the laboratory-maintained colonies, from earlier mating trials (Uzakah, 1995). These weevils were always washed thoroughly with distilled water before being used in the experiments. All experiments were conducted between 11 am and 5 pm, that is, two and eight hours respectively after the onset of scotophase in the laboratory, as the activity rhythm for these insects had been shown to be high and virtually uniform during this period (Uzakah, 1995). Duration of observation for each bioassay was 10 min.

These general conditions were rigidly adhered to throughout these investigations, unless otherwise stated. The experiments included the following:

Responses to freeze-killed weevils

Prior to commencement of experiments, the weevils were thoroughly washed with distilled water and sorted according to sex, using the methods of Longoria (1968) (rostrum punctuations) and Roth and Willis (1963) (curvature of the last abdominal sternite). Weevils of both sexes were freeze-killed individually at -20°C for 30 min, and each was pinned singly through its prothorax into Petri dish (9.5 cm diameter) containing moist sand. Responders (live male or live female weevils) were then introduced singly, so that each Petri dish contained a pair of weevils (a decoy and a live weevil) and the reactions of the live weevils to the decoys were observed. The Petri dishes were covered with similar dishes that had perforations on them to allow for air circulation. Plasticene was used to ensure firm covering for each Petri dish and its cover. The expected responders' reactions were visits to the decoy, arrestments, sniffing (a common practice observed from the weevil's mating behaviour studies, Uzakah, 1995; Uzakah and Odebiyi, 2015) and copulatory attempts.

Each combination (that is, responses of males to male decoys, males to female decoys, females to male decoys and female to female decoys) was replicated eight times (Table 1), and in each case the reactions of responders were recorded. The durations for each observation in this case was 30 min, and no single responder was used more than once.

Responses to body washes/extracts

Twenty females were immersed in 4 ml of n-hexane for between 24, 48 or 168 h, and the extracts obtained were bioassayed against responding males in the dark room. Sixteen to 37 replications were made (Table 2a). Subsequent extractions were made from both males and females (20 of each sex) using dichloromethane for 24 h (Table 2b). The male and female extracts were then bioassayed against both male and female responders in the dark room, such that all the four possible combinations of weevil responses were investigated, that is, males to male extracts, male to female extracts, females to male extracts and females to female extracts. Each treatment was replicated twenty times (Table 2b).

For all these bioassays, a 10 cm diameter Perspex glass arena with walls 9 cm high was placed on a flat, circular glass plate, the floor of which was lined with Whatman's filter paper. At two opposite ends around the periphery of the arena, were placed cut strips (1.5 cm^2) of filter paper, and underneath these were aluminium foil strips of similar dimensions (Plate 1).

One hundred microlitres of test extract and of the control (blank dichloromethane) were applied to these opposite strips of paper respectively and a responding weevil was introduced centrally in the arena, for the determination of its preferred site (that is, its net movement, to either the treated or control site) in each 10-minute bioassay. The aluminium foil strips underneath the strips of paper prevented rapid losses of extracts by seepage through the filter paper, thus ensuring the extracts availability at such sites. In each bioassay, the test extract, blank solvent (or control) and the sex of the responders were unknown to the observer in order to eliminate bias. The order of bioassays was also completely randomized, so as to eliminate positional effects.

Table 1. Responses of male and female banana weevils, *C. sordidus* to their freeze-killed conspecifics, with duration of arrestments (in seconds) in parenthesis.

Bioassay	No. trials	Number of responders			
		Arrested	Mounting	Sniffing	Copulatory attempts
M-M[1]	8	4(8 - 58 s)	0	0	0
M-F[2]	8	3(10 - 451 s)	0	0	0
F-M[3]	8	4(6 - 92 s)	1	0	0
F-F[4]	8	3(12 - 45 s)	1	0	0

[1]Male response or reaction to freeze-killed male weevil (dummy); [2]Male response or reaction to freeze-killed female weevil (dummy); [3]Female response or reaction to freeze-killed male weevil (dummy); [4]Female response or reaction to freeze-killed female weevil (dummy).

Table 2a. Responses of male banana weevils, *C. sordidus* to conspecific female body extracts in n-hexane.

DOE[1]	n	Mean no. of extra[2]	No. of trials			Preferred sites[3]		
		Visits to treatment	Trt	Ctl	Ratio	+ve	-ve	df[4]
7	37	0.6***	78	56	1.4	17	3	20
2	16	1.8**	72	44	1.6	10	1	11
1	20	1.1*	47	25	1.9	12	1	13

*P< 0.05; **P < 0.01; ***P < 0.001.Non-parametric Wilcoxon paired sign rank test; [1]Days of extraction (20 females per 4 ml of n-hexane); [2](Number of visits to treatment) minus (Number of visits to control) divided by (Number of trials, that is, n); [3] The 'net movement' of a responder for each 10-minute bioassay. The overall preference for a treated or control site for each bioassay is expressed under a +ve or –ve site respectively; [4] The effective degree of freedom used in the analysis, after subtracting trials with non-visits (zero visits) and/or trials with ties (even number of visits to trt and ctrl) from the total number of trials (n).

Table 2b. Responses of male and female banana weevils, *C. sordidus* to overnight extraction of conspecific males and females in dichloromethane.

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	Ratio	+ve	-ve	df
Conspecific males								
Males	20	1.2**	44	20	2.2	12	2	14
Females	20	0.9*	43	25	1.7	8	3	11
Conspecific females								
Males	20	1.1**	44	22	2.0	16	2	18
Females	20	-0.4^{ns}	49	56	0.9	7	7	14

*P < 0.05; ** P < 0.01; ns = non significant at 5%; non-parametric Wilcoxon paired sign rank test. Trt = Treatment (female body extracts); Ctl = Control (blank solvent of n-hexane).

After each bioassay, the strips of paper and foil, together with the filter paper that lined the arena floor were discarded. The Perspex glass arena and the basal glass plate were thoroughly washed with soap, rinsed with distilled water and then allowed to dry. New and clean set of materials were used in each bioassay.

Responses to live weevils (olfactometer studies)

Responses to volatiles emanating from live weevils were studied in a still-air olfactometer, the design of which was slightly modified from that of Phillips and Burkholder (1981) and Budenberg et al. (1993). It consisted of a Perspex glass ring (14 cm diameter, and 9 cm high) placed on a flat, circular glass plate which had two holes (each 1 cm^2 in diameter). Each hole was 2.5 cm away on either side of the centre. Directly underneath these holes were placed two small vials (each approximately 2 cm deep), one containing a live insect plus moist cotton wool, and the other moist cotton wool only (control) (Plate 2). Each responder was released at a point equidistant (ca. 6 cm) from the holes and a glass plate was then immediately placed across the Perspex glass ring. The responder's preference for these holes was then determined in each 10-minute bioassay; replicated 20 times (Table 3a). Trial was also repeated with vials holding 5 weevils (emitters) and tested against 5 responders (replicated 20 times) (Table 3b).

The small vials were at the end of each bioassay neatly covered

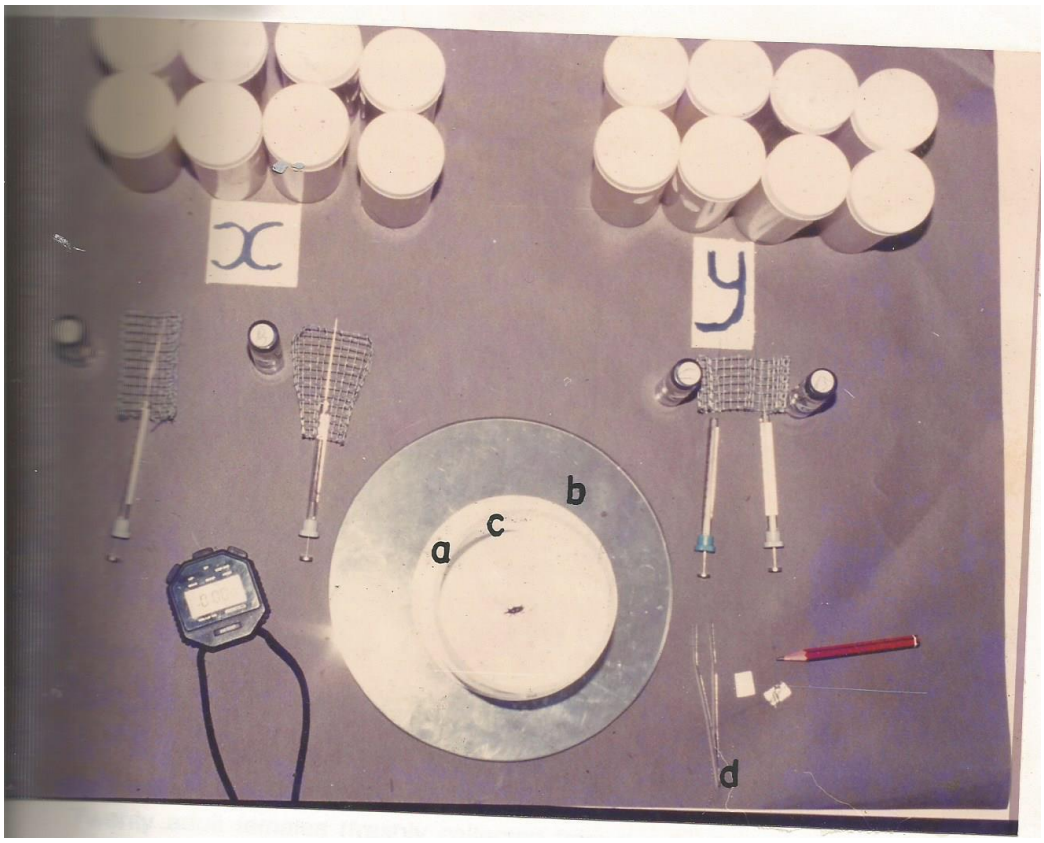

Plate 1. The experimental set-up for bioassaying extracts of the banana weevil, *C. sordidus* in the laboratory. x = responding weevils (males); y = responding weevils (females); a = the glass ring arena; b = the basal glass plate of the arena; c =filter paper placed underneath the arena; d = a pair of soft forceps.

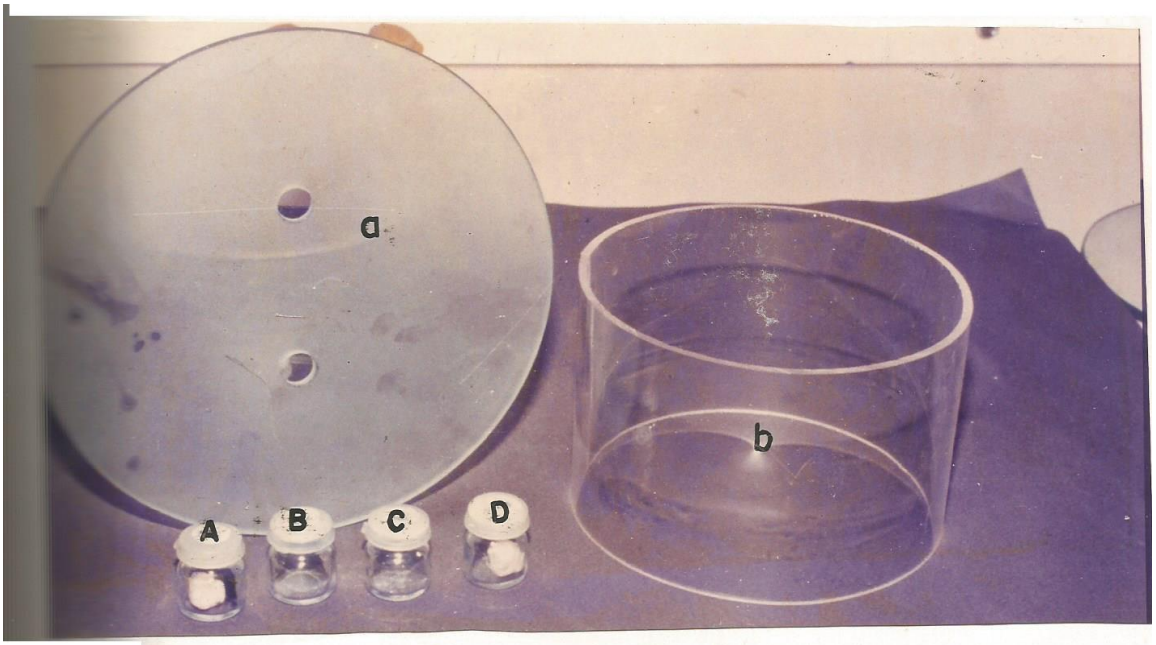

Plate 2. A Close-up of the olfactometer set-up used for the study of the banana weevil, C. sordidus pheromones in the laboratory. a= perforated basal glass plate of arena, b= the glass ring arena, A, B, C, D = glass containers for emitting weevils.

Table 3a. Responses of male and female banana weevils, *C. sordidus* to their conspecifics (using 1 responder against 1 emitter).

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	ratio	+ve	-ve	df
		Conspecific males						
Males	20	0.3^{ns}	7	2	3.5	5	1	6
Females	20	0.6*	18	6	3.0	5	1	6
		Conspecific females						
Males	20	0.4^{ns}	11	4	2.8	6	1	7
Females	20	0^{ns}	5	5	1.0	2	3	5

* P < 0.05; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test.

Table 3b. Responses of male and female banana weevils, *C. sordidus* to their conspecifics (using 5 responders against 5 emitters).

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	ratio	+ve	-ve	df
		Conspecific males						
Males	20	0.1^{ns}	35	33	1.1	8	9	17
Females	20	1.8***	51	15	3.4	15	2	17
		Conspecific females						
Males	20	1.0*	40	20	2.0	13	4	17
Females	20	0.3^{ns}	29	34	0.9	6	8	14

*P < 0.05; *** P < 0.001; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test.

Table 4a. Responses of male and female banana weevils, *C. sordidus* to trapped adult volatiles of their conspecifics[a].

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	ratio	+ve	- ve	df
		Conspecific males						
Males	20	1.8***	56	21	2.7	16	2	18
Females	20	2.1***	56	15	3.7	13	1	14
		Conspecific females						
Males	20	0.9**	37	20	1.9	11	1	12
Females	20	0.7^{ns}	41	27	1.5	7	4	11

** P < 0.01; *** P < 0.001; ns = non significant at 5%; Non-parametric Wilcoxon paired sign rank test. [a] trapping set-up contained moist cotton wool.

with their plastic caps, while the glass rings, basal and cover glass plates were thoroughly washed with soap and distilled water.

Responses to trapped volatiles of field collected adults

Fresh field-collected adults were immediately washed and sexed and 20 of each sex were respectively put into two 0.4 L cylindrical glass flasks which contained damp cotton wool. Air from a compressed air cylinder was passed through the flasks (200 ml/min for 24 to 48 h) via glass tubes (8 × 0.4 cm internal diameter) each of which contained approximately 10 g of activated charcoal plus glass wool.

These glass tubes containing the activated charcoal and glass wool were placed at both ends of each glass flask. The contents of these capillary tubes at the inlet of this set-up cleaned the air entering into the flask, while the ones at the outlet trapped the volatiles from the weevils (replicated 15-20 times) (Table 4a and b). Trapped volatiles were then eluted with 4 ml of dichloromethane (Aldrich, HPLC grade), and the extracts obtained were then bioassayed against responding males and females using the same procedures and set-up as in (2) above. The experiment was repeated with trapped adult volatiles collected from similar sets of weevils but from cylindrical glass flasks or trapping-chambers which contained no damp cotton wool. Trials were replicated 15 to 20 times (Tables 4b and a respectively).

Table 4b. Responses of male and female banana weevils, *C. sordidus* to trapped volatiles of their adult conspecifics:[a]

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	ratio	+ve	-ve	df
		Conspecific males						
Males	15	1.1*	38	22	1.7	10	3	13
Females	15	1.6**	43	19	2.3	9	2	11
		Conspecific females						
Males	15	0.9*	40	27	1.5	9	2	11
Females	15	0.4^{ns}	24	18	1.3	7	5	12

* P < 0.05; *** P < 0.001; ns = non significant at 5%. Non-parametric Wilcoxon paired sign rank test; [a] Trapping set-up without moist cotton wool.

Table 5. Responses of mature male and female banana weevils, C. sordidus to trapped volatiles of their immature conspecifics:[a]

Responders	n	Mean no. of extra	No. of trials			Preferred sites		
		Visits to treatment	Trt	Ctl	ratio	+ve	-ve	df
		Immature conspecific males						
Mature males	15	0.5^{ns}	24	16	1.5	5	2	7
Mature females	15	0.3^{ns}	26	22	1.2	6	5	11
		Immature conspecific females						
Mature males	15	1.4*	48	27	1.8	8	2	10
Mature females	15	0.1^{ns}	16	15	1.1	4	3	7

* P < 0.05; ns = non significant at 5% level; Non-parametric Wilcoxon paired sign rank test; [a]Traping set-up without moist cotton wool.

Responses to trapped volatiles of immature adults

Immature or newly emerged adults (ages between 1 - 10 days old) collected from suckers, were quickly washed and sexed. Twenty females and twenty males were next respectively put into two 0.4 ml cylindrical glass flasks, according to sex, and their volatiles were trapped as above (4). The immature weevils were very tender and delicate, so the trapping duration here was reduced to 24 h only, and no damp or cotton wool was introduced in the chamber, in order to prevent entanglement/concealment in the wool, during the 'brief' trapping period.

Extracts of these immature-adults' volatiles were bioassayed against responding mature males and females, using the same procedures and set-up as in (2) and (4) above; replicated 15 times (Table 5).

Gas chromatography (GC) studies

Extracts of trapped volatiles of freshly collected field adults, and also from the immature weevils (using 20 weevils per 4 ml dichloromethane, in each case) were used in the Gas chromatographic (GC) studies). For each extract three microlitres (3 µl) was injected into the GC (Hewlett Packard 5890) using a fused silica capillary column (SPBTM-1)(30 m × 0.32 mm × 0.25 µm film thickness). The temperature program used was: initial temperature 40°C for two minutes, 5°C/minute rise to the final temperature 250°C and final time 20 min. Actual dilute extracts (unconcentrated) were used in this study as the concentrated ones produced too many peaks.

RESULTS

Responses to freeze-killed weevils

The responders' reactions to freeze-killed weevils are presented in Table 1. The expected behavioural reactions from responders (particularly of males to freeze-killed females) were arrestments, mounting, sniffing or copulatory attempts (or genitalia contacts). However, no records of sniffing or copulatory attempts were made in this study. There were few instances of female climbing over the dummies, but there was nothing to suggest that males were in any way sexually attracted or aroused by the presence of these dummies, since they failed to sniff or to attempt mating with these dummies. The few arrestments that live males made around female dummies did not result in any distinct behavioural activity. The arrestments recorded for the different treatments were not in any way different from one another (Table 1). The longest period of arrestment of 451 s by male

responder to freeze-killed female, did not even result in mounting, sniffing or copulatory attempts.

Responses to body washes/extracts

The results of male responders to female body extracts are shown in Table 2a while Table 2b gives the responses of both males and females to body extracts of males and of females.

There was no significant response of males to the female body extracts. The response seemed stronger with increase in days of extraction (DOE) as indicated by the decreasing level of probability with increasing DOE (Table 2a). Table 2b however, showed that in addition to the significant male responses to female extracts, both males and females responded significantly to male body extracts. Female responders, however, did not respond significantly to female body extracts.

Responses to live weevils (olfactometer studies)

Females responded significantly ($P < 0.001$) to 'concealed' live males. Similarly, males responded significantly ($P < 0.01$) to 'concealed' females. However, there was no significant response from males to 'concealed' males and from females to 'concealed' females (Table 3).

Responses to trapped adult volatiles

Males responded significantly to the trapped adult volatiles of both males and females, however, females responded significantly to only the trapped adult male volatiles and not to the trapped adult female volatiles (Table 4a and b).

Responses to trapped volatiles of immature adults

Males responded significantly to trapped volatiles of immature females. The responses of males to the trapped volatiles of immature males, and of females to the trapped volatiles of immature males or immature females were not significant (Table 5).

Gas chromatographic (GC) studies

The GC profiles of the banana weevil volatiles are shown in Figures 1 and 2. The figures clearly showed these compounds to be relatively volatile, since some peaks were recorded within the first five minutes of start of the run (that is, at such low temperature of 40°C). Combined gas chromatography and electroantennogram (GC-EAG) studies however, could not be done to establish the physiologically active peaks.

DISCUSSION

Our results strongly suggest that two pheromones are produced in this insect - a female produced sex pheromone and a male produced aggregation pheromone. The apparent absence of discreet behavioural responses (sniffing, copulatory attempts) by responding males to freeze-killed females suggest that at death, female banana weevils cease to be sexually attractive to the males.

This could be due to cessation in the production of the mating stimulant (pheromone) by females at death, or sudden loss of it soon after death of the female. This pheromone may be relatively volatile, as can be seen from the chromatograms (Figures 1 and 2), so it is likely to be lost after death. The male indifference to the female could, however, also be due to the absence of a specific (and perhaps a complementary) movement by the freeze-killed (still) females, perhaps necessary for mating in this insect. Dean et al. (1969) reported that in the tsetse fly, *Glossina morsitans orientalis* Vanderplank (Diptera: Glossinidae) the male flies appeared sexually activated only after movement of the female. Langley et al (1975) similarly reported that in the laboratory, the male *G. m. morsitans* Vanderplank was aroused by movement of other individuals. They, contrary to the results obtained in this study, also reported that dead tsetse flies of this species were sexually attractive to mature males and that the attractiveness of the females was not diminished by low temperature storage or vacuum drying. Selander (1978) and Tiles et al. (1988) similarly reported repeated matings with freeze-killed females in the pine weevils.

The fact that a male virtually had to contact a female, and sniff her abdominal tip with his antennae in order to perceive the pheromone (Uzakah, 1995; Uzakah and Odebiyi, 2015), suggests that the female pheromone in *C. sordidus* is only active within a very short range. This observation seem to be in agreement with that of Cross and Mitchell (1966), who suggested that female boll weevils produced a weak secondary pheromone which males perceived by olfactory means over distances of less than 5 cm.

The observed lack of strong behavioural responses (e.g. sniffing, copulatory attempts, entanglements etc.) to freeze-killed males by responders, as was usually observed with live weevils, also seem to suggest that males at death, similarly cease production of the pheromone (aggregation pheromone), and that the pheromone is also a volatile and not a contact pheromone.

The more significant responses observed by responding males to extracts obtained from prolonged exposures (48 and 168 h of extraction) of the female to the solvent than to those obtained from short extraction

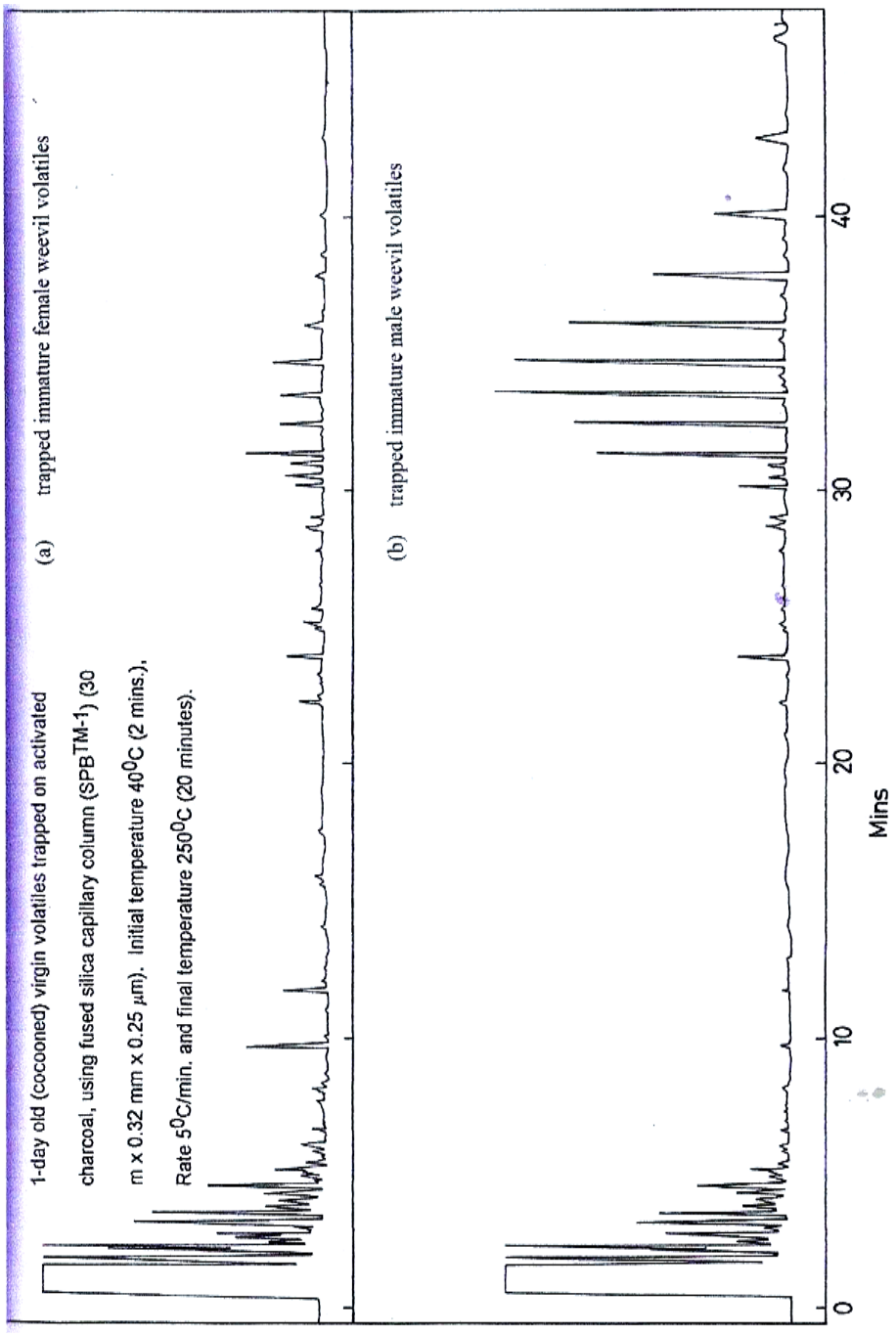

Figure 1. Gas chromatographic profiles of trapped volatiles of immature banana weevils, *C. sordidus.*

period (24 h) (Table 1) confirm that the pheromone is produced internally and so not present on the surface of the female (that is, a volatile and not a contact pheromone). Perhaps the more thorough extraction resulted in the dissolution of internal lipids and facilitated the extraction of internal sources (which were not possible with the less thorough extraction). Further evidence of this is obtained from the various responses of responders to trapped volatiles of mature and immature adults, and also from the still-air olfactometer studies, where significant responses by live males to live females were observed.

The significant responses by responders (males and females) to males in all these bioassays (except to the freeze-killed males) are consistent with the view that the males of this insect produced a volatile aggregation

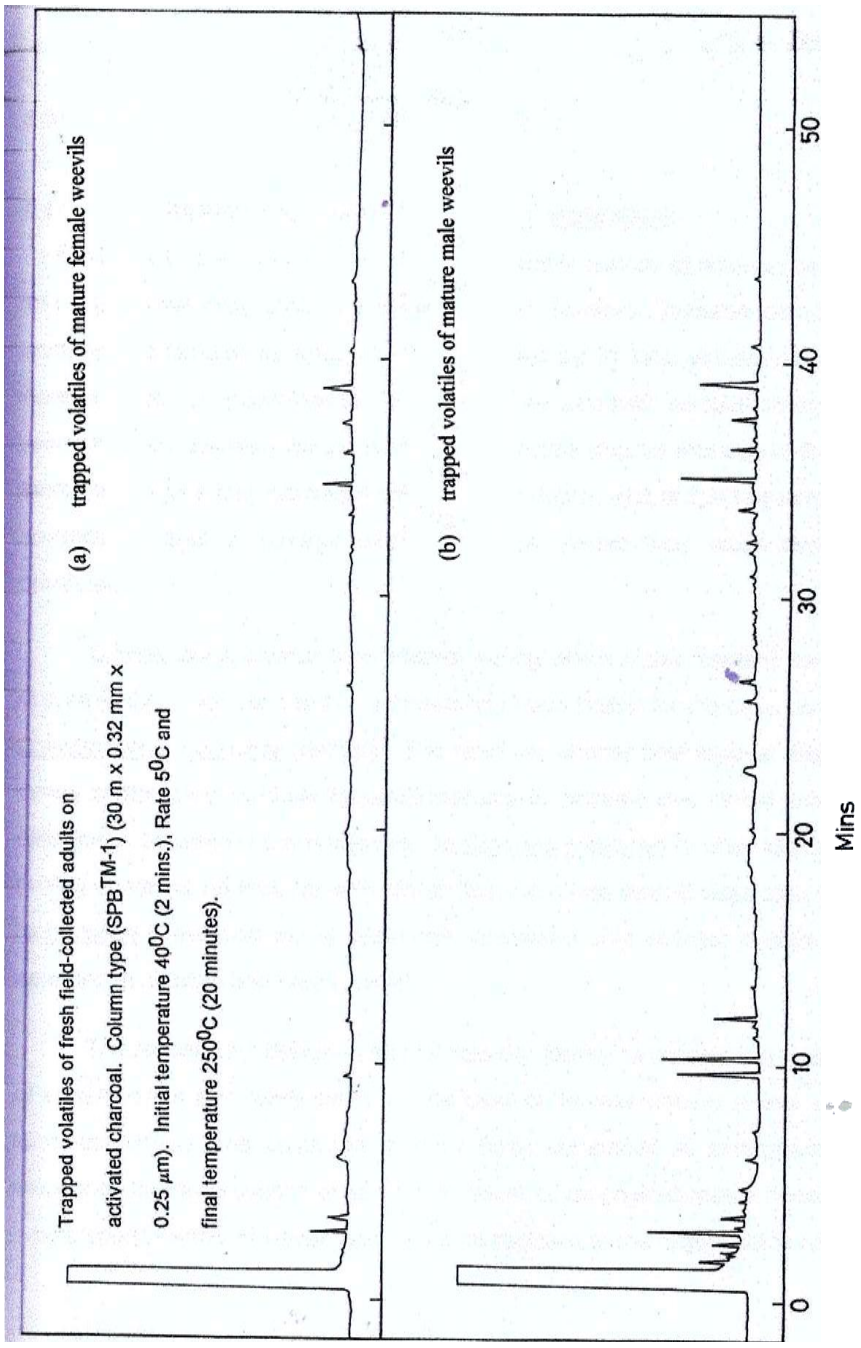

Figure 2. Gas chromatographic profiles of trapped volatiles of adult banana weevils, *C. sordidus.*

pheromone. However, the failure of earlier workers to detect the female sex pheromone was probably due to the fact that the female pheromone is produced in small amounts. Short range pheromones are common in insects. In the rove beetle (Coleoptera: Staphylinidae), Peschke (1983) reported that the cuticular pheromone was detected only over a short distance of 2.2 mm, but that the reception mechanism was by olfaction. Humphries (1967) reported that the mating behavior of the hen flea, *Ceratophyllus gallinae* (Schrank) Insecta: Siphonaptera), is only initiated if the male came into contact with female and received some specific stimulus from her abdomen through his maxillary palps.

The presence of two types of pheromones (a male produced aggregation pheromone and a female sex pheromone) has been reported in other weevils (Tinzaara

et al., 2002). For instance, Selander (1978) and Tiles et al. (1986) confirmed the presence of two types of pheromones in the pine weevil, *Hylobius abietis* (L), while the findings of Phillips and Burkholder (1981), and Sharma and Deora (1980) showed this for the rice weevil, *Sitophilus oryzae* (L). Hedin et al. (1979) also confirmed this for the boll weevil, *Anthonomus grandis* Boheman.

The female pheromone of the banana weevil, compared to that of the male was apparently a weak one, and so only effective over a short range and essentially for mating purposes; while the male one, necessary for aggregating the sexes was evidently more effective over longer ranges. A similar observation was made by McKibben et al. (1977) who reported that male boll weevils produced 1 µg of the pheromone per day while the females produced approximately 0.01 µg per day, confirming the weakness of the female weevil pheromones. The finding was supported by later work by Hedin et al. (1979) for this weevil. According to these authors, this was perhaps the reason for the failure of earlier workers to find the pheromone of the female weevil.

The significant responses made by adult males to trapped volatiles from immature females (approximately 1 - 10 DAE), and the apparent lack of responses by responders to those of immature males (Table 5) suggests that the female pheromone is produced at an earlier stage than that of the male during the life of the insect. The production of these pheromones in these weevils is apparently linked with age at sexual maturity. Previous laboratory studies (Uzakah, 1995), reveal that female *C. sordidus*, were not sexually mature until about 2 weeks after emergence. It would be interesting to investigate the ages at which these weevils commenced the production of these pheromones.

Selander (1978) and Tiles et al. (1988) observed that virgin females of the pine weevils, *Hylobius abietis* (Coleoptera: Curculionidae) produced a sex pheromone that attracted males. They also observed a strong attraction between virgin males and adult males, and so inferred that males at this stage perhaps produced the sex pheromone. Attraction between immature males and adult males of the banana weevils was also observed in this study, but it was not found to be statistically significant (Table 5).

Comparisons of the GC profiles for the trapped volatiles of immature weevils (that is, male vs. female; Figure 1); and also those of mature weevils (males vs females; Figure 2) clearly revealed qualitative differences - the males in both cases produced higher peaks for the compounds that were common to both sexes. Combined gas chromatography and electroantennogram (GC-EAG) studies could not be done to ascertain the physiologically active peaks in these chromatograms, neither was GC-MS (that is, gas chromatography cum mass spectrometry) studies embarked upon to help characterize the peaks found in these chromatograms.

The male aggregation pheromone of *C. sordidus* has since been isolated, synthesized and even field-tested (Tinzaara et al., 2011, Alpizar et al., 2012). This synthetic pheromone, named sordidin or Cosmolure+ (depending on manufacturers), have been found to be successful in mass trappings; capturing 18 times more weevils than the conventional pseudostem traps in Uganda (Tinzaara et al., 2011); although Alpizar et al (2012) highlighted a range of 2½ to 8-fold increases under different conditions.

Sex pheromones in like manner, are also widely and successfully used against several crop pests particularly, against lepidopterous pests of fruits, vegetables and forests (Srivastava and Dhaliwal, 2012). Mason and Jansson (1991) even reported its potential for the Coleoptera (that is, as a mating disruptant in the sweet potato weevil, *Cylas formicarius* (Coleoptera: Apionidae). Same may be applicable with the banana weevil sex pheromone, and thus help to bring about delays, reductions or even prevent propagation of the weevil's population. This potential benefit of the banana weevil sex pheromone, if tested and proven, may be combined with sordidin plus other good cultural practices for a more holistic, novel and effective control of this serious pest of Musa.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research

Apocarotenoid gene expression in saffron (*Crocus sativus* L.)

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Saffron (*Crocus sativus* L.) is a triploid sterile plant characterized by its long red stigmas, which produce and store its chief carotenoid derivatives-safranal, crocin and picrocrocin. Saffron selections of Kashmir are heterogeneous for floral characteristics which are mainly attributed to the environmental factors, though genetic factors may have role with regard to its differential characteristics across various selections found in the area. Identification of high yielding selections using the existing gene pool of saffron may help in improving the productivity of this crop. The present study was conducted at Biotechnology Lab, Central Institute of Temperate Horticulture, Srinagar, India during 2012-2013 to find out the apocarotenoid gene expression in thirty-one morphogically distinct clones of saffron. Comparative apocarotenoid gene expression through Real Time PCR analysis revealed a significant variation in Zeaxanthin cleavage dioxygenase (*CsZCD*) and lycopene-β-cyclase (*CsLYC*) genes between the most divergent selections (CITH-S-107 and PAM-S-116) indicating a possible role of these genes for regulating the apocarotenoid production in stigma. Significant variation was observed with respect to stigma length (2.86 to 4.84 cm) across thirty-one selected saffron clones. Although some clones showed variation with respect to stigma number, in addition to normal trifid stigmas, some clones produce tetrafid stigmas as well but this character was not heritable. Our study provides sufficient knowledge to identify clones with better stigma characteristics and higher apocarotenoid biosynthetic potential for further crop improvement programs.

Key words: *Crocus sativus*, *CsZCD*, *CsLYC*, RT-PCR, RNA isolation, apocarotenoid.

INTRODUCTION

Crocus sativus unknown as a wild plant is considered to be a mutant that has derived from *C. cartwrightianus.* The cultivated clone was probably selected for its triploid vigour and extra long stigmas and has been maintained in cultivation for over 3000 years. The dried red stigmas of *C. sativus*, has been used as flavouring and colouring agent since then and is currently considered the world's most expensive spice. The major components of saffron are the apocarotenoids cis- and trans-crocins, picrocrocin (β-D-glucopyranoside of hydroxyl-β-cyclocitral), and its degradation product, the odour-active safranal (Kanakis et al., 2004; Sanchez and Winterhalter, 2013). Clonal

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selection plays an important role with reference to improving the traits of cultivated saffron. Both experimental mutagenesis and procedures aimed at doubling a chromosome set of saffron have not lead to encouraging results (Nehvi et al., 2007a, b). Therefore, superior clones showing better stigma characteristics viz., higher stigma length, increased number of stigmas and higher apocarotenoid biosynthetic potential needs to be selected and mass multiplied. Furthermore, such genotypes need to be analyzed in detail to find out the active principal behind their superiority, which can be exploited for saffron crop improvement. Apocarotenoids have been extensively studied in the stigma tissue because of their organoleptic properties. It has been proposed that the biogenesis of the main colour principles, crocins, and the odour active compound, safranal is derived by bio-oxidative cleavage of zeaxanthin (Pfander and Schurtenberger, 1982) by a 7, 8 (7', 8') cleavage reaction. Apocarotenoid biosynthetic pathway possesses many enzymes which are catalyzing the reactions and coded by related key genes such as *PSY*, *LYC*, *CCD*, *BCH* and *ZCD*. Beta-carotene with two rings is built up via cyclization of lycopene with lycopene-β-cyclase (*LYC*). The hydroxylation of β-carotene in MVA pathway is catalyzed by β-carotenoid hydroxylase that coded by *BCH* gene to yield zeaxanthin (Castillo et al., 2005). The biogenesis of the color and odor active compounds of saffron are derived by bio-oxidative cleavage of zeaxanthin (Rubio-Moraga et al., 2009; Gomez-Gomez et al., 2010) at the points 7, 8 (7', 8') by zeaxanthin cleavage dioxygenase (*CsZCD*) to produce crocetin dialdehyde and picrocrocin. In *C. sativus* stigmas, the final step involves glucosylation of the generated zeaxanthin cleavage products by glucosyltransferase 2 enzyme which is coded by *CsUGT2* gene in chromoplast of stigmas and then sequestered into the central vacuole of the fully developed stigmas (Bouvier et al., 2003).

MATERIALS AND METHODS

Thirty-one (31) different saffron clones representing the core collection for saffron germplasm conservation were used in the study. These clones were maintained at Central Institute of Temperate Horticulture, Srinagar (J&K), India. Stigmas were collected from September to November 2013. Freshly cut stigmas were quickly immersed in liquid nitrogen and then stored at -80°C for RNA isolation, while dried stigmas were used for apocarotenoid extraction.

Morphological parameter

The stigma length was measured by digital caliper. Data on stigma length are means of five replicates of 10 flowers. The data was analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using SPSS for windows (v. 15. SPSS Inc USA).

RNA extraction and cDNA preparation

Frozen stigmas were ground in cold and sterilized mortar and pestle into fine powder and total RNA was extracted using RNA isolation kit (Roche Applied Science "Penzberg, Germany") following the manufacturer's protocol. Quality of the extracted RNAs was checked by measuring the absorbance at 260 and 280 nm using Nano-Drop and RNAs with ratio of OD 260/280 ranging from 1.2 to 1.5 were used for cDNA synthesis. For first-strand cDNA synthesis, 5 µg of total RNA template and 18-bp oligo dT primer and cDNA synthesis kit (Roche Applied Science "Penzberg, Germany") were used as described by the manufacturer. The cDNA was stored at -20°C until use.

The concentration of RNA was determined using the Beer-Lambert law, which predicts a linear change in absorbance with concentration. An A260 reading of 1.0 is equivalent to about 40 µg/ml of RNA and the OD at 260 nm is used to determine the RNA concentration in a solution. RNA has its absorption maximum at 260 nm and the ratio of the absorbance at 260 and 280 nm is used to assess the RNA purity of an RNA preparation. Pure RNA has an A260/A280 of 2.1. The NanoDrop® ND-1000 UV-Vis Spectrophotometer enables highly accurate analyses of extremely small samples with remarkable reproducibility. The sample retention system eliminates the need for cuvettes and capillaries, which decrease the amount of sample, required for the measurement.For apocarotenoid extraction, saffron stigmas were extracted with methanol (100 mL) in a microcentrifuge tube for 5 min on ice. Tris-HCl (50 mM, pH 7.5; containing 1 M NaCl) was then added (100 mL) and incubated for 10 min on ice. The precipitate was collected by centrifugation at 3,000 g for 5 min at 4°C. The pellet was then reground in acetone (400 mL) and incubated on ice for 10 min. The mixture was centrifuged at 3,000 g for 5 min at 4°C. This step was repeated until no color was detected in the pellet. The supernatants were pooled and evaporated and the dried residues were stored at -80°C.

RT- PCR analysis

Reverse transcription was carried for amplification of *CsZCD* and *CsLYC* with Cs*TUB* gene as internal control, using AMVRT cDNA kit (Roche Applied Science, "Penzberg, Germany") according to user manual. RNA isolated from all the samples was quantified spectrophotometrically and equal concentration of RNA was used for semi-quantitative comparison of gene expression through reverse transcription polymerase chain reaction. RNA was handled under RNase free environment and all operations were carried out using -20°C cooler. Forward and reverse primers sequences used for amplification of these genes and expected length of amplicons are shown in Table 1. PCR reactions were performed in thermocycler (Takara, Japan) with 2 to 5 µg of cDNA. Initial denaturizing at 95°C for 5 min followed by 35 cycles of amplification according to the subsequent scheme; denaturizing 1 min at 94°C, annealing at 56.2°C for 30 s and extension at 72°C for 40 s and final extension at 72°C for 7 min. The experiments were repeated twice. Subsequently 5 µl of the PCR products were used on 1.2% (w/v) agarose (Sigma-Aldrich, St Louis, MO, USA).

Real time PCR analysis

The Real Time PCR was performed in 96-well plates with a LightCycler 480 real-time PCR instrument (Roche Diagnostics) using the LightCycler 480 SYBR Green I Master kit. SYBR Green I is a DNA double-strand-specific dye. During each phase of DNA synthesis, the SYBR Green I dye, which is included in the reaction mix, binds to the amplified PCR products; the amplicon can be detected by its fluorescence. Hot start protocols used with the

Table 1. Sequence and amplicon size of primers used for real time PCR analysis.

Primer	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	Amplicon size (bp)
CsZCD	GTCTTCCCCGACATCCAGATC	CTCTATCGGGCTCACGTTGG	241
CsLYC	AGATGGTCTTCATGGATTGGAG	ATCACACACCTCTCATCCTCTTC	247
CsBCH	TCGAGCT TCGGCATCACATC	GCAATACCAAACAGCGTGATC	495
CsGT2	GATCTGCCGTGCGTTCGTAAC	GATGACAGAGTTCGGGGCCTTG	400
CsTUB	TGATTTCCAACTCGACCAGTGTC	ATACTCATCACCCTCGTCACCATC	225

LightCycler 480 SYBR Green I Master have been shown to significantly improve the specificity, sensitivity, and yield of PCR. Heat-labile blocking groups on some of the amino acid residues of FastStart Taq DNA Polymerase make the modified enzyme inactive at room temperature. Therefore, there is no elongation during the period when primers can nonspecifically bind. The FastStart Taq DNA Polymerase is "activated" by removing the blocking groups at a high temperature (that is, a pre-incubation step at +95°C for 5 min).

Reactions were performed in triplicate and contained 5 µl SYBR Green I Master, 2 µl PCR-grade water, 2 µl cDNA, and 0.5 µl of each of the 10 µM forward and reverse gene-specific primers in a final volume of 10 µl. The reactions were incubated at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 56.2°C for 15 s, and 72°C for 20 s. In our study reporter system, the intercalating SYBR green assay was used. SYBR green binds to all double-stranded DNA via intercalation between adjacent base pairs. When bound to DNA, a fluorescent signal is emitted following light excitation.

As amplicon numbers accumulate after each PCR cycle, there is a corresponding increase in fluorescence. Post-PCR dissociation (melting) curve analysis (60 to 95°C) was carried out to confirm that the fluorescence signal is generated only from target templates and not from the formation of nonspecific PCR products. LightCycler 480 software (version 1.5; Roche Diagnostics) was used to collect the fluorescence data. Advanced relative quantification between the genotypes and three stages of stigma development were done through $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen, 2001).

RESULTS AND DISCUSSION

Average stigma length varies from 2.86 cm in selection PAM-S-116 to 4.84 cm in CITH-S-107 (Table 2), which is higher than reported earlier by Caiola (2004) and Nehvi et al. (2007a). Nehvi et al. (2007a) reported stigma length range of 2.41 to 3.87 in 438 random selections of saffron from Kashmir. Stigma length variation was successfully transferred to next generation; hence this is a stable inherited trait hence can be tagged with molecular markers for association studies. The results are in accordance with the erlier reports of Mir et al. (2012b). Genetic variation and heritability of agro-morphological and phytochemical traits in saffron populations have been studied, populations were found significantly different for most evaluated traits like leaf number per plant, leaf length, fresh stigma characteristics, dry stigma weight per plot, spathe number and the content of crocins, picrocrocin and safranal (Baghalian et al., 2010).

RT-PCR for semi quantitative expression of *CsZCD* and *CsLYC* genes between two distinct genotypes (CITH-S-107 and PAM-S-116) was done during scarlet stage of stigma development. *CsTUB* was used as an internal control. These genotypes did not show significant variation, however, *CsZCD* gene showed higher expression than *CsLYC* gene (Figure 1). Although, reverse transcription PCR analysis is the semi-quantitative method of gene expression but it is very good method to find an idea about comparative level of gene expression and is a highly sensitive and specific method useful for the detection of rare transcripts or for the analysis of samples available in limiting amounts. RT-PCR is increasingly used to detect small changes in gene expression that would otherwise be undetectable (Freeman et al., 1999).

Quantitative real time PCR (Q-PCR) approaches combine the detection of target template with quantification by recording the amplification of a PCR product via a corresponding increase in the fluorescent signal associated with product formation during each cycle in the PCR. Quantification of gene (or transcript) numbers is determined during the exponential phase of the PCR amplification when the numbers of amplicons detected are directly proportional to the initial numbers of target sequences present within the environment. Quantification of the target gene during exponential amplification avoids problems that are associated with so-called 'end-point' PCR (in which amplicons are only analyzed after completion of the final PCR cycle) (Smith and Osborn, 2009).

In *C. sativus,* the development of the stigmas occurs concomitantly with transition of amyloplasts to chromoplasts and parallel with biosynthesis and accumulation of apocarotenoid which relates to expression levels of *CsZCD* and *CsLYC* gene (Bouvier et al., 2003; Rubio-Moraga et al., 2009). The pattern of accumulation of the apocarotenoid crocetin, picrocrocin and the different crocins in developed saffron stigmas was investigated by extracting stigmas from two selections (CITH-S-107 and PAM-S-116) with diverse crocin contents and stigma length (Tables 2 and 3). Biogenesis of crocetin glucosides and picrocrocin are initiated by zeaxanthin cleavage dioxygnase, which is coded by *CsZCD* gene (Rubio-Moraga et al., 2009). Although, carotenoid accumulation and composition during stigma development of *C. sativus* is highly regulated by the coordinated transcriptional activation of

Table 2. Variability in stigma length of different saffron (*Crocus sativus*) selections.

Selections	Stigma length (cm)
CITH-S-125	3.74^{k}±0.04
CITH-S-123	4.38^{n}±0.06
CITH-S-124	3.86^{k}l±0.05
CITH-S-122	3.98^{l}±0.04
CITH-S-12	3.44^{j}±0.05
CITH-S-121	4.14^{m}±0.05
CITH-S-107	4.84^{o}±0.02
CITH-S-120	3.86^{kl}±0.05
CITH-S-104	3.72^{k}±0.04
CITH-S-117	3.3^{hij}±0.03
CITH-S-112	3^{abcde}±0.06
CITH-S-113	3.16^{efgh}±0.05
CITH-S-119	2.98^{abcd}±0.04
CITH-S-118	3.22^{ghi}±0.07
CITH-S-10	2.9^{ab}±0.05
CITH-S-103	3.04^{bcdef}±0.04
CITH-S-43	3.16^{efgh}±0.05
CITH-S-114	3.3^{hij}±0.11
CITH-S-115	3.2^{fghi}±0.05
CITH-S-105	3.08^{cdefg}±0.04
PAM-S-106	3.34^{ij}±0.09
PAM-S-102	3.14^{defgh}±0.08
PAM-S-108	3.4^{j}±0.03
PAM-S-11	3.3^{hij}±0.03
PAM-S-116	2.86^{a}±0.03
PAM-S-13	3.42^{j}±0.05
PAM-S-101	3.7^{k}±0.03
PAM-S-3	3.3^{hij}±0.03
PAM-S-111	3.12^{defg}±0.07
BUD-S-110	2.92^{abc}±0.04
BUD-S-76	3.2^{fghi}±0.03

Means followed by the same letter within the columns are not significantly different (P = 0.05) using DMRT.

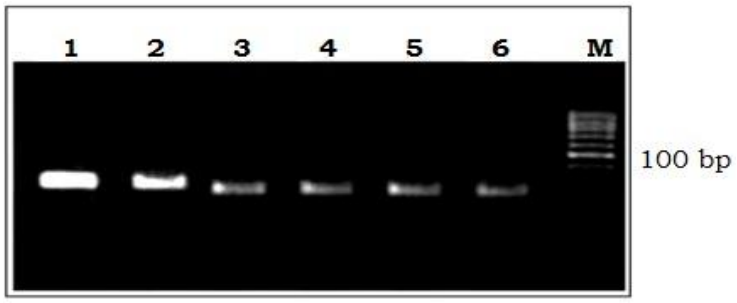

Figure 1. Reverse Transcription PCR for semi-quantitative analysis for CsTUB (Lane 1 and 2), *CsLYC* (Lane 3 and 4) and *CsZCD* (Lane 5 and 6) between PAM-S-116 and CITH-S-107 genotypes respectively.

Table 3. Apocarotenoid quantification through HPLC in 31 *Crocus sativus* L. genotypes.

Accession/Clone	Crocin (mg/g)	Safranal (mg/g)	Picrocrocin (mg/g)
CITH-S-125	45^{e}±0.88	0.24^{efg}±0.011	1.12^{cdefg}±0.006
CITH-S-123	42^{abc}±1.15	0.22^{bcde}±0.005	1.08^{bcde}±0.07
CITH-S-124	43^{bcde}±0.57	0.26^{gh}±0.00	1.03^{bcd}±0.08
CITH-S-122	41^{ab}±0.57	0.27^{hi}±0.003	1.03^{bcd}±0.08
CITH-S-12	41^{ab}±0.57	0.24^{efg}±0.01	1.08^{bcde}±0.03
CITH-S-121	42^{abc}±0.57	0.23^{def}±0.003	1.14^{cdefg}±0.04
CITH-S-107	45de±1.15	0.22^{bcde}±0.005	1.20^{defg}±0.01
CITH-S-120	41^{ab}±1.15	0.21^{abc}±0.008	1.10^{bcdef}±0.009
CITH-S-104	42^{abcd}±0.33	0.23^{cdef}±0.005	1.16^{cdefg}±0.02
CITH-S-117	43^{bcde}±0.57	0.24^{efg}±0.005	0.87^{a}±0.06
CITH-S-112	43^{bcde}±0.57	0.25^{fg}±0.005	1.11^{cdefg}±0.05
CITH-S-113	42^{abc}±1.15	0.27^{hi}±0.005	1.13^{cdefg}±0.05
CITH-S-119	44^{cde}±1.15	0.23^{cdef}±0.005	1.10^{bcdef}±0.02
CITH-S-118	40^{a}±1.15	0.28^{i}±0.003	1.04^{bcd}±0.04
CITH-S-10	42^{ab}c±0.00	0.24^{efg}±0.011	1.17^{cdefg}±0.007
CITH-S-103	41^{ab}±0.57	0.21^{abc}±0.005	1.22^{efg}±0.012
CITH-S-43	43^{bcde}±0.57	0.23^{cdef}±0.005	1.01^{abc}±0.007
CITH-S-114	42^{abcd}±0.33	0.21^{abc}±0.005	1.25^{efg}±0.007
CITH-S-115	41^{abc}±0.33	0.22^{bcde}±0.003	1.27^{f}±0.007
CITH-S-105	42^{abc}±0.57	0.20^{ab}±0.003	1.26^{ef}±0.012
PAM-S-106	43^{bcde}±1.15	0.21^{bcd}±0.006	1.01^{abc}±0.007
PAM-S-102	44^{cde}±1.15	0.27^{hi}±0.003	1.14^{cdefg}±0.012
PAM-S-108	40^{a}±0.57	0.28^{i}±0.003	1.00^{abc}±0.11
PAM-S-11	41^{abc}±0.33	0.26^{gh}±0.005	1.12^{cdefg}±0.012
PAM-S-116	40^{a}±1.15	0.23^{def}±0.003	0.94^{ab}±0.07
PAM-S-13	43^{bcde}±1.15	0.22^{bcde}±0.003	1.17^{cdefg}±0.018
PAM-S-101	44^{cde}±1.15	0.19^{a}±0.003	1.24^{efg}±0.012
PAM-S-3	41^{ab}±0.57	0.22^{bcde}±0.005	1.16^{cdefg}±0.012
PAM-S-111	42^{abcd}±0.33	0.21^{bcd}±0.008	1.00^{abc}±0.11
BUD-S-110	41^{ab}±0.00	0.23^{cdef}±0.005	1.15^{cdefg}±0.03
BUD-S-76	43^{bcde}±0.57	0.24^{efg}±0.005	1.15^{cdefg}±0.02

Means followed by the same letter within the columns are not significantly different (P = 0.05) using DMRT.

carotenoid biosynthetic genes (Castillo et al., 2005), the expression levels did not parallel the accumulation of the apocarotenoid compounds, suggesting that the formation of these compounds is controlled at a different level, such as carotenoid cleavage dioxygenase expression (Rubio-Moraga et al., 2008; Baghalian et al., 2010). Since, zeaxanthin cleavage being an important step in apocarotenoid accumulation, the authors choosed *CsZCD* gene expression to compare two divergent selections in saffron. The second gene studied in this experiment was *CsLYC*. Lycopene β-cyclase, the product of *CsLYC* gene, catalyzes the cyclization of lycopene to β-catrotene.

β-catrotene and zeaxanthin are the important precursors of saffron apocarotenoid in MVA pathway. Reverse transcription and real time PCR was performed with RNA purified from stigmas between two genotypes. Real Time PCR amplification of *CsZCD*, *CsLYC* and Tubulin genes between CITH-S-107 and PAM-S-116 at scarlet stages of stigma development is shown in Figure 2. PAM-S-116 showed 98 and 81% relative expression of gene *CsZCD* and *CsLYC*, respectively, to that of CITH-S-107 selection. *CsZCD* showed 3 fold higher expressions over *CsTUB* gene in both of the genotypes whereas *CsLYC* showed 0.7 and 0.3 fold expression over *CsTUB* in CITH-S-107 and PAM-S-116, respectively.

These results are in close agreement with the earlier reports of Mir et al. (2012a) who also reported 2.69 folds relative increase in CsZCD gene expression over tubulin gene expression in scarlet stage and 0.90 and 0.69 folds decrease in CsZCD gene expression over tubulin gene expression in orange and yellow stages, respectively. Increased expression of *CsZCD* gene with corresponding increase in apocarotenoid content during the

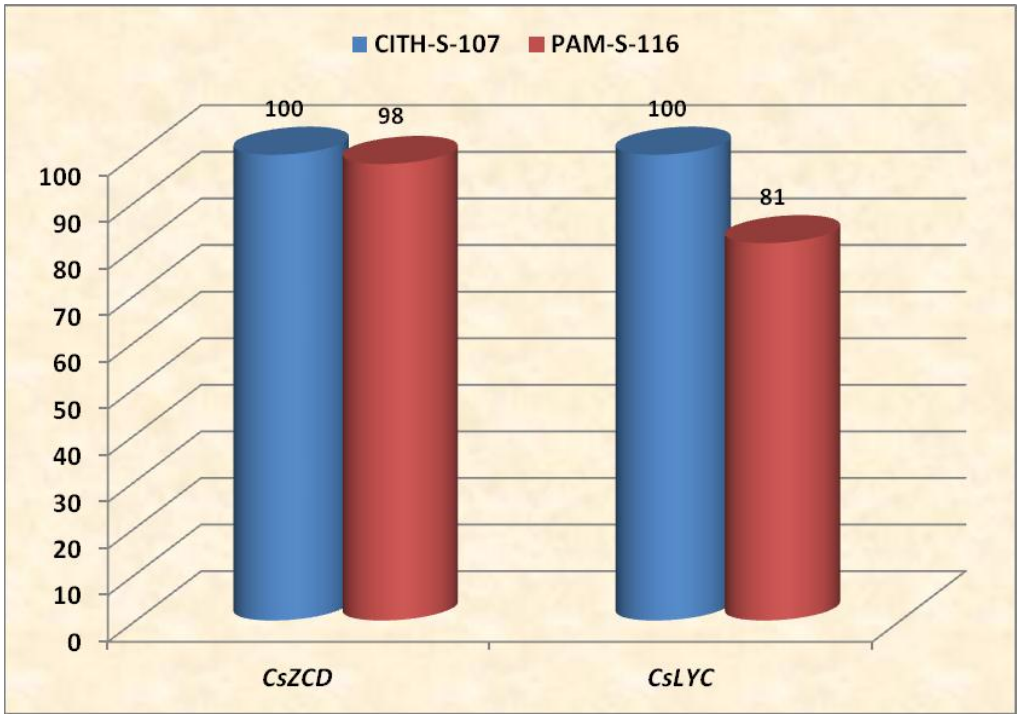

Figure 2. Comparative gene expression of *CsZCD* and *CsLYC* between CITH-S-107 and PAM-S-116 genotypes through Real Time PCR.

development of stigma, suggests its regulatory role in apocarotenoid biosynthesis and stigma development in saffron (Mir et al., 2012a, b). Our results showed that variation in apocarotenoid content may be due to different expression levels of *CsZCD* and *CsLYC* genes between the genotypes.

Conflict of Interest

The authors have not declared any conflict of interest.

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Scientific Research and Essays

Full Length Research Paper

The time delayed feedback control to suppress the vibration of the autoparametric dynamical system

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The response of a dynamical system of two-degree-of-freedom with parametrically excited pendulum is solved and studied. The delayed feedback control is applied to suppress or stabilize the vibration of the system. The case of 1:2 sub-harmonic resonances between pendulum and primary system is studied; the method of multiple scales is applied to obtain second-order approximations of the response of the system. It is shown that the delayed feedback control can be used to suppress the vibration or stabilize the system when the saturation control is invalid, the vibration of the system can be suppressed by the delayed feedback control. The effect of delay on the suppression is discussed; the vibration of the system can be suppressed at some values of the delay.

Key words: Frequency response, delayed feedback control, multiple times scale, vibration suppression.

INTRODUCTION

In the last years, many papers have been devoted to the control of resonantly forced systems in various engineering fields. In passive vibration absorbers a physical device is connected with the primary structure, while in the case of active absorbers the device is replaced by a control system of sensors, actuators and filters. Active control of mechanical and structural vibrations is superior to passive control, because the former is more flexible in many aspects.

Periodically forced nonlinear systems under delay control have been analyzed by Plaut and Hsieh (1987) in the case of nonlinear structural vibrations with a time delay in damping. The studying of an approach for implementing an active nonlinear vibration absorber (El-Bassiouny, 2005). The strategy exploits the saturation phenomenon that is exhibited by multi-degree-of-freedom systems with cubic nonlinearities possessing one-to-one internal resonance. The proposed technique consists of introducing a second- order controller and coupling it to the plant through a sensor and an actuator, where both the feedback and control signals are cubic. The vibration and chaos control of nonlinear torsion al vibrating systems is studied (El-Bassiouny, 2006).

The resonance, stability and chaotic vibration of a quarter-car vehicle model with time-delay feedback is investigated in (Naik and Singru, 2011). The primary, super harmonic and sub harmonic resonances of a harmonically excited nonlinear quarter-car model with

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time delayed active control are investigated. They focused on the influence of time delay in the system. This naturally gives rise to a delay differential equation model of the system. It was found that proper selection of time-delay showed optimum dynamical behavior. The modeling and optimal active control with time delay dynamics of a strongly nonlinear beam. They investigated the control by a sandwich beam and one using piezoelectric absorber (Nana Nbendjo et al., 2003, 2009).

The effect of the time delay on the non-linear control of a beam when subjected to multi-external excitation forces is determined, multiple scale perturbation method is applied to obtain the solution up to the second order approximations (El-Gohary and El-Ganaini, 2011). Using the delayed feedback control and saturation control to suppress the vibration of the dynamical system under external force is studied (Zhao and Xu, 2012). The nonlinear dynamical analysis on four semi-active dynamic vibration absorbers with time delay is solved (Yongjun and Mehdi, 2013). Vibration reduction, stability and resonance of a dynamical system excited by external and parametric excitations via time-delay absorber is studied (Sherif and Sayed, 2014). The dynamical system of a twin-tail aircraft, which is described by two coupled second order nonlinear differential equations having both quadratic and cubic nonlinearities, solved and controlled (Amer et al., 2009). The effect of different controllers on the vibrating system and the saturation control of a linear absorber to reduce vibrations due to rotor blade flapping motion is obtained (Sayed and Kamel, 2011, 2012). studied the primary resonance, stability and design methodology of a piecewise bilinear system under cubic velocity feedback control with a designed time-delay are investigated through combining multi-scale perturbation method with Fourier expansion, the effects of time delay on dynamics behaviors are explored (Gao and Chen, 2013).

The nonlinear analysis of time-delay position feedback control of container cranes studied (Nayfeh and Baumann, 2008). Stabilizability of the turning process subjected to a digital proportional- derivative controller is analyzed, the governing equation involves a term with continuous-time point delay due to the regenerative effect and terms with piecewise-constant arguments due to the zero-order hold of the digital control (Lehotzky et al., 2014). The dynamical system with time varying stiffness subjected to multi external forces studied. The system is written as two degree of freedom consists of the main system and absorber. The multiple time scale perturbation method is applied to get the approximate solution up to the third approximation. The stability of the system at the simultaneous primary resonance is investigated using both frequency response equations and phase-plane methods (Amer and Ahmed, 2014). The application of the renormalization group (RG) methods to the delayed differential equation is determined by analyzing the Mathieu equation with time delay feedback, get the amplitude and phase equations, and then obtain the approximate solutions by solving the corresponding R G equations (Wu and Xu, 2013).

In this paper, the delayed feedback control is applied to suppress the vibration of the system. The equation of motion and the perturbation analysis and the stability of the equilibrium solutions are given. All possible resonance cases are extracted and investigated at this approximation order. The stability of the system is studied using the phase plane and frequency response curves. The analytical solutions and numerical simulations of the delayed feedback control are presented and compared.

EQUATIONS OF MOTION

A two degree-of-freedom dynamic vibration absorber system with a parametrically excited pendulum is described in (Song et al., 2003). In the present paper, a position feedback with delay is introduced into the model to control the vibration of the system and the model is shown in Figure 1. The system consists of the mass M, the linear spring with stiffness k, and the viscous damper presented by coefficient c. The second system is a simple pendulum of mass m_p hinged at the system. The distance between the supporting point and the center of gravity of the pendulum is l. J and c_θ represent the inertia moment with respect to the supporting point and the damping coefficient of the pendulum respectively. The system is excited directly by a harmonic force $f(t) = Fx\cos(\omega t)$. a time delayed position feedback $g_1[x(t-\tau)-x(t)]$ is introduced into the auto parametric dynamic vibration absorber system. The equations of motion of the delayed feedback control system are

$$(M+m_p)\frac{d^2x}{dt^2}+c\frac{dx}{dt}+kx+m_pl(\frac{d^2\theta}{dt^2}\sin\theta+(\frac{d\theta}{dt})^2\cos\theta) + g_1(x_\tau - x) = f(t) \quad (1)$$

$$J\frac{d^2\theta}{dt^2}+c_\theta\frac{d\theta}{dt}+m_pl\sin\theta(g+\frac{d^2x}{dt^2})=0, \quad (2)$$

where x the displacement of is M, θ is the angle of rotation of the pendulum, and t, $x_\tau = x(t-\tau)$. It should be noted that the delayed feedback disappears in (1) and (2) when $\tau = 0$, and (1) and (2) are identical to Song et al. (2003). Thus it is easy to observe effects of the delayed feedback on vibration suppression performance when $\tau \neq 0$.

Firstly, a set of dimensionless (or normalized) variables are defined as:

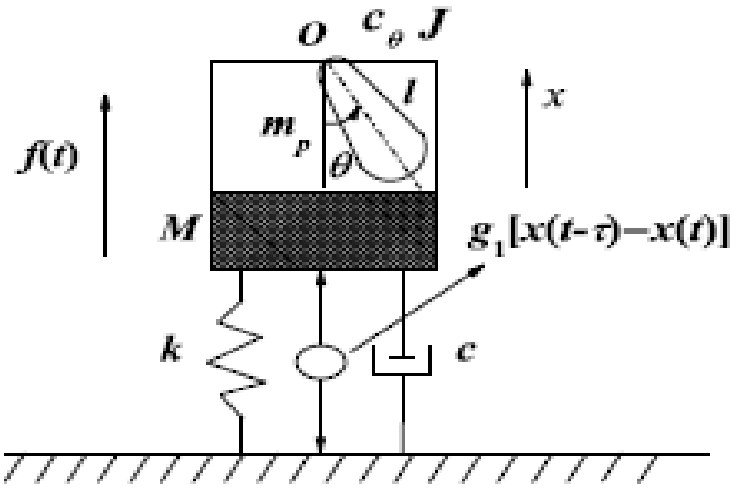

Figure 1. A model describing the autoparametric dynamical vibration absorber with delayed feedback.

$$t^* = \frac{\omega}{\Omega}\mathrm{t},\ \tau^* = \frac{\omega}{\Omega}\tau,\ \eta = \frac{x}{l},\ F = \frac{p_0}{kl},\ R = \frac{m_p}{M},\ \alpha = \frac{\omega_1}{\omega_3},\ \beta = \frac{\omega_2}{\omega_1},\ \gamma = \frac{\omega_4}{\omega_3},\ \mu = \frac{m_p l^2}{J},$$

$$\zeta_1 = \frac{c}{2M\,\omega_3(1+R)},\ \zeta_2 = \frac{c_\theta}{2J\,\omega_3},\ \omega_1 = \sqrt{\frac{k}{M+m_p}},\ \omega_2 = \sqrt{\frac{g}{l}},\ \omega_3 = \sqrt{\frac{k}{M}},$$

$$\omega_4 = \sqrt{\frac{g_1}{M+m_p}},\ \Omega = \frac{\omega}{\omega_3}.$$

Equations (1) and (2) can be written as the following non-dimensional forms by dropping the asterisk for convenience.

$$\eta'' + G_1\eta' + \Omega_1^2\eta + G_2(\theta''\sin\theta + \theta'^2\cos\theta) + H_3(\eta_\tau - \eta) = G_3\eta\cos(\Omega t), \tag{3}$$

$$\theta'' + H_1\theta' + (\Omega_2^2 + H_2\eta'')\sin\theta = 0, \tag{4}$$

where $\Omega_1^2 = \alpha^2$, $\Omega_2^2 = \mu\alpha^2\beta^2$, $G_1 = 2\zeta_1$, $H_1 = 2\zeta_2$, $G_2 = \frac{R}{1+R}$, $H_2 = \mu$, $G_3 = F\alpha^2$, $H_3 = \gamma^2$, Ω is dimensionless frequency, $\eta_\tau = \eta(t-\tau)$, $()' = \frac{d()}{dt^*}$.

Equations (1) and (2) are the nonlinear delayed differential equation of the forced vibration system. The non-autonomous differential equations can be transformed into autonomous differential equations by the method of average or the multiple scales. The method of multiple scales is used to obtain the approximate analytical solutions. The bifurcation problem is investigated based on the new autonomous differential equations given thus.

PERTURBATION ANALYSIS

Since θ are very small, here it is set $\sin\theta \approx \theta - \frac{\theta^3}{6}$ and $\cos\theta \approx 1 - \frac{\theta^2}{2}$. In the following part, the method of multiple scales is employed in the perturbation analysis. A small dimensionless perturbation parameter ε $(0 < \varepsilon < 1)$ is introduced into the equations used for bookkeeping only. A fast scale is characterized in $T_0 = t$ with the motion at Ω and a slow scale in $T_1 = \varepsilon t$. It can be set that $\eta = \varepsilon\hat{\eta}$, $\theta = \varepsilon\hat{\theta}$, $F = \varepsilon\hat{F}$, $\zeta_1 = \varepsilon\hat{\zeta}_1$, $\zeta_2 = \varepsilon\hat{\zeta}_2$, $\gamma^2 = \varepsilon\hat{\gamma}^2$, and $\eta_\tau = \varepsilon\hat{\eta}_\tau$ in

order to have the damp, nonlinear terms, and Sub-harmonic resonance force appear in the same perturbation magnitude. Dropping the ' ^ ' for convenience, (3) and (4) are written as:

$$\eta'' + \Omega_1^2\eta = \varepsilon[-G_1\eta' - \mathrm{G}_2(\theta''\theta + \theta'^2) + \mathrm{G}_3\eta\cos(\Omega t) - H_3(\eta_\tau - \eta)] + \varepsilon^3\,\mathrm{G}_2(\theta''\frac{\theta^3}{6} + \theta'\frac{\theta^2}{6}) \quad (5)$$

$$\theta'' + \Omega_2^2\theta = \varepsilon[-H_1\theta' - H_2\theta\eta''] + \varepsilon^2\Omega_2^2\frac{\theta^3}{6} + \varepsilon^3 H_2\eta''\frac{\theta^3}{6} \quad (6)$$

The method of multiple scales is employed to seek second order approximate solutions of Equations (5) and (6) in the following form:

$$\eta(t,\varepsilon) = \eta_0(T_0,T_1) + \varepsilon\eta_1(T_0,T_1) + \varepsilon^2\eta_2(T_0,T_1) + O(\varepsilon^3) \quad (7)$$

$$\eta_\tau(t,\varepsilon) = \eta_{0\tau}(T_0,T_1) + \varepsilon\eta_{1\tau}(T_0,T_1) + \varepsilon^2\eta_{2\tau}(T_0,T_1) + O(\varepsilon^3) \quad (8)$$

$$\theta(t,\varepsilon) = \theta_0(T_0,T_1) + \varepsilon\theta_1(T_0,T_1) + \varepsilon^2\theta_2(T_0,T_1) + O(\varepsilon^3) \quad (9)$$

The derivatives with respect to time are expressed in terms of the new scales as:

$$\frac{d}{dt} = D_0 + \varepsilon D_1\,, \qquad \frac{d^2}{dt^2} = D_0^2 + 2\varepsilon D_0D_1 + \varepsilon^2 D_1^2, \quad (10)$$

Where $D_k = \dfrac{\partial}{\partial T_k},\ k = 0,1.$

Substituting Equations (7) to(10) into Equations (5) and (6) and equating coefficients of like powers of ε yield that:

Order ε^0:

$$\left(D_0^2 + \Omega_1^2\right)\eta_0 = 0 \quad (11)$$

$$\left(D_0^2 + \Omega_2^2\right)\theta_0 = 0 \quad (12)$$

Order ε^1:

$$\left(D_0^2 + \Omega_1^2\right)\eta_1 = -2D_0D_1\eta_0 - G_1D_0\eta_0 - G_2\theta_0D_0^2\theta_0 - G_2(D_0\theta_0)^2 + G_3\eta_0\cos(\Omega T_0) - H_3\eta_{0\tau} + H_3\eta_0 \quad (13)$$

$$\left(D_0^2 + \Omega_2^2\right)\theta_1 = -2D_0D_1\theta_0 - H_1D_0\theta_0 - H_2\theta_0D_0^2\eta_0 \quad (14)$$

Order ε^2:

$$(D_0^2 + \Omega_1^2)\eta_2 = -D_1^2\eta_0 - 2D_0D_1\eta_1 - G_1D_1\eta_0 - G_1D_0\eta_1 - G_2\theta_1D_0^2\theta_0 - 2G_2\theta_0D_0D_1\theta_0 - G_2\theta_0D_0^2\theta_1 - 2G_2D_0\theta_0D_1\theta_0 - 2G_2D_0\theta_0D_0\theta_1 + G_3\eta_1\cos(\Omega T_0) - \mathrm{H}_3(\eta_{1\tau} - \eta_1) \quad (15)$$

$$(D_0^2 + \Omega_2^2)\theta_2 = -D_1^2\theta_0 - 2D_0D_1\theta_1 - H_1D_1\theta_0 - H_1D_0\theta_1 - 2H_2\theta_0D_0D_1\eta_0 - H_2\theta_0D_0^2\eta_1 - H_2\theta_1D_0^2\eta_0 + \frac{1}{6}\Omega_2^2\theta_0^3 \quad (16)$$

The general solution of Equations (11) and (12) can be expressed as:

$$\eta_0(\mathrm{T}_0,\mathrm{T}_1) = A(\mathrm{T}_1)\exp(i\,\Omega_1T_0) + cc \quad (17)$$

$$\theta_0(\mathrm{T}_0,\mathrm{T}_1) = B(\mathrm{T}_1)\exp(i\,\Omega_2T_0) + cc \quad (18)$$

Where A and B are arbitrary functions at this level of approximation, cc denotes complex conjugate. The external excitation and the delayed feedback are expressed in complex forms.

$$G_3\cos(\Omega T_0) = \frac{1}{2}G_3\exp(i\,\Omega T_0) + cc \quad (19)$$

$$\eta_{0\tau}(\mathrm{T}_0,\mathrm{T}_1) = A_\tau(\mathrm{T}_1)\exp(i\,\Omega_1(T_0 - \tau)) + cc \quad (20)$$

A_τ can be expanded in a Taylor series (2002) under the assumption that the product of time delay and the small parameter ε is small compared to unity.

$$A_\tau(\mathrm{T}_1) = A(\mathrm{T}_1 - \varepsilon\tau) = \mathrm{A}(\mathrm{T}_1) - \varepsilon\tau\frac{dA(\mathrm{T}_1)}{dt} + \frac{1}{2}\varepsilon^2\tau^2\frac{d^2A(\mathrm{T}_1)}{dt^2} + \ldots \quad (21)$$

Substituting Equations (17) to (21) into Equations (13) and (14) yields

$$\left(D_0^2 + \Omega_1^2\right)\eta_1 = (-2i\,\Omega_1D_1A - G_1i\,\Omega_1A + H_3A)\exp(i\,\Omega_1T_0) + \frac{1}{2}G_3A\exp(i\,(\Omega_1 + \Omega)T_0) + 2G_2B^2\Omega_2^2\exp(2i\,\Omega_2T_0) + \frac{1}{2}G_3\bar{A}\exp(i\,(\Omega - \Omega_1)T_0) - H_3A\exp(i\,\Omega_1(T_0 - \tau)) + \mathrm{cc} \quad (22)$$

$$\left(D_0^2 + \Omega_1^2\right)\eta_1 = (-2i\,\Omega_1D_1A - G_1i\,\Omega_1A + H_3A)\exp(i\,\Omega_1T_0) + H_2\Omega_1^2\,\mathrm{AB}\exp(i\,(\Omega_1 + \Omega_2)T_0) + H_2\Omega_1^2\,\mathrm{A\bar{B}}\exp(i\,(\Omega_1 - \Omega_2)T_0) + cc \quad (23)$$

The particular solutions of the above equations are:

$$\eta_1(T_0,T_1) = A_1\exp(i\,\Omega_1T_0) + E_1\exp(2i\,\Omega_2T_0) + \mathrm{E}_2\exp(i\,(\Omega_1 + \Omega)T_0) + E_3\exp(i\,(\Omega - \Omega_1)T_0) + \mathrm{cc} \quad (24)$$

$$\theta_1(T_0,T_1) = B_1 \exp(i\,\Omega_2 T_0) + E_4 \exp(i\,(\Omega_1+\Omega_2)T_0) + E_5 \exp(i\,(\Omega_1-\Omega_2)T_0) + \text{cc} \quad (25)$$

Substituting Equations (17), (18), (24) and (25) into Equations (15) and (16), hence solving the resulting equations, we get:

$$\eta_2(T_0,T_1) = E_6 \exp(2i\,\Omega_2 T_0) + \mathrm{E}_7 \exp(i\,(\Omega_1+\Omega)T_0) + E_8 \exp(i\,(\Omega-\Omega_1)T_0) + \mathrm{E}_9 \exp(i\,(\Omega_1+2\Omega_2)T_0) + \mathrm{E}_{10} \exp(i\,(\Omega_1-2\Omega_2)T_0) + \mathrm{E}_{11} \exp(i\,(2\Omega_2+\Omega)T_0) + \mathrm{E}_{12} \exp(i\,(2\Omega_2-\Omega)T_0) + \mathrm{E}_{13} \exp(i\,(\Omega_1+2\Omega)T_0) + \mathrm{E}_{14} \exp(i\,(2\Omega-\Omega_1)T_0) + A_2 \exp(i\,\Omega_1 T_0) + \text{cc} \quad (26)$$

$$\theta_2(T_0,T_1) = E_{15} \exp(i\,(\Omega_1+\Omega_2)T_0) + E_{16} \exp(i\,(\Omega_1-\Omega_2)T_0) + E_{17} \exp(3i\,\Omega_2 T_0) + \mathrm{E}_{18} \exp(i\,(\Omega+\Omega_1+\Omega_2)T_0) + \mathrm{E}_{19} \exp(i\,(\Omega+\Omega_1-\Omega_2)T_0) + \mathrm{E}_{20} \exp(i\,(\Omega-\Omega_1+\Omega_2)T_0) + \mathrm{E}_{21} \exp(i\,(\Omega-\Omega_1-\Omega_2)T_0) + \mathrm{E}_{22} \exp(i\,(2\Omega_1+\Omega_2)T_0) + \mathrm{E}_{23} \exp(i\,(2\Omega_1+\Omega_2)T_0) + \mathrm{E}_{24} \exp(i\,(2\Omega_1-\Omega_2)T_0) + B_2 \exp(i\,\Omega_2 T_0) + \text{cc} \quad (27)$$

Where A_i, $\mathrm{B}_i, (\mathrm{i}=1,2)$ and $\mathrm{E}_n, (n=1,...,24)$ are complex functions in T_1 and cc denotes complex conjugate. From the above derived solutions, the reported resonance cases are:

1) Primary resonance: $\Omega \cong \Omega_1$.

2) Sub-harmonic resonance: $\Omega \cong 2\Omega_1$.

3) Internal resonance: $\Omega_1 \cong \Omega_2,\ \Omega_1 \cong 2\Omega_2$.

4) Combined resonance: $\Omega \cong \pm(2\Omega_2-\Omega_1),\ \Omega \cong (2\Omega_2+\Omega_1)$.

5) Simultaneous resonance: any combination of above resonance cases is considered as simultaneous resonance.

STABILITY ANALYSIS

From numerically studying the different resonance cases, we find that the worst is resonance case is the simultaneous resonance case $\Omega \cong 2\Omega_1,\ \Omega_1 \cong 2\Omega_2$. So that we introduce the detuning parameters σ_1 and σ_2 according to the following:

$$\Omega = 2\Omega_1 + \varepsilon\sigma_1,\ \Omega_1 = 2\Omega_2 - \varepsilon\sigma_2 \quad (28)$$

Substituting Equation (28) into Equations (22) and (23) and setting the coefficients of the secular terms to zero yield the solvability conditions given by:

$$-2i\,\Omega_1 D_1 A - G_1 i\,\Omega_1 A + \frac{1}{2}G_3 \bar{A} \exp(i\,\sigma_1 T_1) + 2G_2 B^2 \Omega_2^2 \exp(i\,\sigma_2 T_1) - H_3 A \exp(-i\,\Omega_1 \tau) + H_3 A = 0 \quad (29)$$

$$-2i\,\Omega_2 D_1 B - H_1 i\,\Omega_2 B + H_2 \Omega_1^2 A\bar{B} \exp(-i\,\sigma_2 T_1) = 0 \quad (30)$$

We express the complex function A, B in the polar form as

$$A = \frac{1}{2}a \exp(i\,\theta_1),\ B = \frac{1}{2}b \exp(i\,\theta_2) \quad (31)$$

Where a, b, θ_1 and θ_2 are real-valued functions. Substituting Equation (31) into Equations (29) and (30) and separating real and imaginary parts yields:

$$a' = -\frac{1}{2}G_1 a - \frac{1}{2\Omega_1}G_2\Omega_2^2 b^2 \sin\varphi_2 + \frac{1}{4\Omega_1}G_3 \mathrm{a}\sin\varphi_1 + \frac{1}{2\Omega_1}aH_3 \sin\Omega_1\tau \quad (32)$$

$$a\varphi_1' = \sigma_1 a + \frac{1}{\omega_1}G_2\Omega_2^2 b^2 \cos\varphi_2 + \frac{1}{2\Omega_1}G_3 \mathrm{a}\cos\varphi_1 - \frac{1}{\Omega_1}aH_3 \cos\Omega_1\tau + \frac{1}{\Omega_1}aH_3 \quad (33)$$

$$b' = -\frac{1}{2}H_1 b + \frac{1}{4\Omega_2}H_2\Omega_1^2 ab \sin\varphi_2 \quad (34)$$

$$b(\frac{\varphi_1'}{4} + \frac{\varphi_2'}{2}) = (\frac{\sigma_1}{4} - \frac{\sigma_2}{2})\mathrm{b} + \frac{1}{4\Omega_2}H_2\Omega_1^2 ab \cos\varphi_2 \quad (35)$$

Where $\varphi_1 = \sigma_1 T_1 - 2\theta_1,\ \varphi_2 = \theta_1 - 2\theta_2 - \sigma_2 T_1$.

For the steady state solution $a' = b' = 0,\ \varphi_m' = 0;\ m = 1,2$. Then it follows from Equations (32) to (35) that the steady state solutions are given by:

$$-\frac{1}{2}G_1 a + \frac{1}{4\Omega_1}G_3 \mathrm{a}\sin\varphi_1 + \frac{1}{2\Omega_1}aH_3 \sin\Omega_1\tau - \frac{1}{2\Omega_1}G_2\Omega_2^2 b^2 \sin\varphi_2 = 0 \quad (36)$$

$$-\sigma_1 a - \frac{1}{2\Omega_1}G_3 \mathrm{a}\cos\varphi_1 + \frac{1}{\Omega_1}aH_3 \cos\Omega_1\tau - \frac{1}{\Omega_1}aH_3 - \frac{1}{\Omega_1}G_2\Omega_2^2 b^2 \cos\varphi_2 = 0 \quad (37)$$

$$\frac{1}{2}H_1 b - \frac{1}{4\Omega_2}H_2\Omega_1^2 ab \sin\varphi_2 = 0 \quad (38)$$

$$(\frac{\sigma_1}{4} - \frac{\sigma_2}{2})\mathrm{b} + \frac{1}{4\Omega_2}H_2\Omega_1^2 ab \cos\varphi_2 = 0 \quad (39)$$

From Equations (36) to (39), we have the following cases:

Case 1. $a \neq 0$ and $b \neq 0$: in this case, the frequency response equation are given by the following equations:

$$(G_1^2+\sigma_1^2+\frac{1}{4\Omega_1^2}G_3^2+\frac{2}{\Omega_1^2}H_3^2-\frac{1}{\Omega_1}G_1G_3\sin\varphi_1 -\frac{2}{\Omega_1}G_1H_3\sin\Omega_1\tau-\frac{1}{\Omega_1^2}G_3H_3\cos(\varphi_1+\Omega_1\tau) -\frac{2}{\Omega_1}\sigma_1H_3\cos\Omega_1\tau+\frac{2}{\Omega_1}\sigma_1H_3+\frac{1}{\Omega_1^2}G_3H_3\cos\varphi_1 -\frac{2}{\Omega_1^2}H_3^2\cos\Omega_1\tau+\frac{1}{\Omega_1}\sigma_1G_3\cos\varphi_1)a^2-\frac{1}{\Omega_1^2}G_2^2\Omega_2^4b^4=0 \quad (40)$$

$$H_1^2b^2+(\frac{\sigma_1}{2}-\sigma_2)^2b^2-(\frac{1}{4\Omega_2^2}H_2^2\Omega_1^4)a^2b^2=0 \quad (41)$$

Case 2: $a \neq 0$ and $b=0$: in this case, the frequency response equation is given by:

$$(G_1^2+\sigma_1^2+\frac{1}{4\Omega_1^2}G_3^2+\frac{2}{\Omega_1^2}H_3^2-\frac{1}{\Omega_1}G_1G_3\sin\varphi_1 -\frac{2}{\Omega_1}G_1H_3\sin\Omega_1\tau-\frac{1}{\Omega_1^2}G_3H_3\cos(\varphi_1+\Omega_1\tau) -\frac{2}{\Omega_1}\sigma_1H_3\cos\Omega_1\tau+\frac{2}{\Omega_1}\sigma_1H_3+\frac{1}{\Omega_1^2}G_3H_3\cos\varphi_1 -\frac{2}{\Omega_1^2}H_3^2\cos\Omega_1\tau+\frac{1}{\Omega_1}\sigma_1G_3\cos\varphi_1)a^2=0 \quad (42)$$

Linear solution

Now to the stability of the linear solution of the obtained fixed let us consider A and B in the forms

$$A(T_1)=\frac{1}{2}(p_1-iq_1)\exp(i\delta T_1),$$

$$B(T_1)=\frac{1}{2}(p_2-iq_2)\exp(i\delta_1 T_1)$$

Where p_1, p_2, q_1 and q_2 are real values and considering

$$\delta=\frac{\sigma_1}{2},\ \delta_1=\sigma_2.$$

Substituting Equation (42) into the linear parts of Equations (29) and (30) and separating real and imaginary parts, the following system of equations are obtained:

For the solution ($a \neq 0$ and $b \neq 0$), we get:

$$p_1'=(-\frac{1}{2}G_1+\frac{1}{2\Omega_1}H_3\sin\Omega_1\tau)p_1+(\frac{1}{4\Omega_1}G_3-\frac{\sigma_1}{2}+\frac{1}{2\Omega_1}H_3\cos\Omega_1\tau-\frac{1}{2\Omega_1}H_3)q_1 \quad (43)$$

$$q_1'=(\frac{1}{2}\sigma_1+\frac{1}{4\Omega_1}G_3-\frac{1}{2\Omega_1}H_3\cos\Omega_1\tau+\frac{1}{2\Omega_1}H_3)p_1+(-\frac{1}{2}G_1+\frac{1}{2\Omega_1}H_3\sin\Omega_1\tau)q_1 \quad (44)$$

$$p_2'=(-\frac{1}{2}H_1)p_2-\sigma_2q_2 \quad (45)$$

$$q_2'=\sigma_2p_2-(\frac{1}{2}H_1)q_2 \quad (46)$$

The stability of the linear solution in this case is obtained from the zero characteristic equation

$$\begin{vmatrix} (-\frac{1}{2}G_1+\frac{1}{2\Omega_1}H_3\sin\Omega_1\tau-\lambda) & (\frac{1}{4\Omega_1}G_3-\frac{\sigma_1}{2}+\frac{1}{2\Omega_1}H_3(\cos\Omega_1\tau-1)) & 0 & 0 \\ (\frac{1}{2}\sigma_1+\frac{1}{4\Omega_1}G_3+\frac{1}{2\Omega_1}H_3(1-\cos\Omega_1\tau)) & (-\frac{1}{2}G_1+\frac{1}{2\Omega_1}H_3\sin\Omega_1\tau-\lambda) & 0 & 0 \\ 0 & 0 & (-\frac{1}{2}H_1-\lambda) & -\sigma_2 \\ 0 & 0 & \sigma_2 & (-\frac{1}{2}H_1-\lambda) \end{vmatrix}=0 \quad (47)$$

After extract we obtain that

$$\lambda^4+r_1\lambda^3+r_2\lambda^2+r_3\lambda+r_4=0, \quad (48)$$

Where $r_1=H_1+G_1-\frac{1}{\Omega_1}H_3\sin\Omega_1\tau$,

$$r_2=\frac{1}{4}H_1^2+\frac{1}{4}G_1^2+\frac{1}{\Omega_1}(\frac{1}{4\Omega_1}H_3\sin\Omega_1\tau-H_1-\frac{1}{2}G_1)H_3\sin\Omega_1\tau +\frac{\sigma_1}{2}(\frac{\sigma_1}{2}+\frac{1}{\Omega_1}H_3)+\sigma_2^2-\frac{1}{16\Omega_1^2}G_3^2+\frac{1}{4\Omega_1^2}H_3^2 -\frac{1}{2\Omega_1}(\sigma_1-\frac{1}{2\Omega_1}H_3\cos\Omega_1\tau+\frac{1}{\Omega_1}H_3)H_3\cos\Omega_1\tau+G_1H_1$$

$$r_3=\frac{1}{4}H_1(\frac{1}{\Omega_1}H_3+\sigma_1)^2-\frac{1}{2\Omega_1}H_1H_3\cos\Omega_1\tau(\frac{1}{\Omega_1}H_3+\sigma_1) +\frac{1}{4\Omega_1^2}H_1H_3^2+\frac{1}{4}H_1G_1^2+(\sigma_2^2+\frac{1}{4}H_1^2)(G_1-\frac{1}{\Omega_1}H_3\sin\Omega_1\tau) -\frac{1}{2\Omega_1}G_1H_1H_3\sin\Omega_1\tau-\frac{1}{16\Omega_1^2}H_1G_3^2$$

$$r_4 = (\frac{1}{4}(\frac{1}{\Omega_1}\mathrm{H}_3+\sigma_1)^2 - \frac{1}{2\Omega_1}H_3\cos\Omega_1\tau(\frac{1}{\Omega_1}\mathrm{H}_3+\sigma_1)+\frac{1}{4}G_1^2 - \frac{1}{16\Omega_1^2}\mathrm{G}_3^2+\frac{1}{4\Omega_1^2}\mathrm{H}_3^2-\frac{1}{2\Omega_1}\mathrm{G}_1\mathrm{H}_3\sin\Omega_1\tau)(\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2)$$

According to the Routh-Huriwitz criterion, the above linear solution is stable if the following are satisfied:

$$r_1 > 0,\ r_1r_2 - r_3 > 0,\ r_3(r_1r_2 - r_3) - r_1^2r_4 > 0,\ r_4 > 0. \quad (49)$$

When Conditions (49) are not satisfied, the initial equilibrium solution is unstable, and bifurcations may occur. But if Conditions (49) are not satisfied.

Conditions (49) imply that all the eigenvalues of the Equation (47) have negative real parts. When Conditions (49) are not satisfied, this is not the case. First, we prove that zero cannot be a simple eigenvalue of the Equation (47). If zero is an eigenvalue of the Equation (47), then it is not simple. In fact, suppose zero is an eigenvalue of the Equation (47), then it follows $r_4 = 0.$

Denote $A = \frac{1}{\Omega_1}\mathrm{H}_3+\sigma_1,$ we have

$$r_4 = (\frac{1}{4}A^2 - (\frac{1}{2\Omega_1}H_3\cos\Omega_1\tau)A+\frac{1}{4}G_1^2 - \frac{1}{16\Omega_1^2}\mathrm{G}_3^2 + \frac{1}{4\Omega_1^2}\mathrm{H}_3^2-\frac{1}{2\Omega_1}\mathrm{G}_1\mathrm{H}_3\sin\Omega_1\tau)(\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2) = 0 \quad (50)$$

Regard A as a variable, if Equation (50) has a real solution, it follows, the discriminate of quadratic Equation (50), $\Delta \geq 0.$ On the other hand,

$$\Delta = \frac{1}{4\Omega_1^2}H_3^2(\cos\Omega_1\tau)^2(\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2)^2 - (\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2)^2(\frac{1}{4}G_1^2 - \frac{1}{16\Omega_1^2}\mathrm{G}_3^2+\frac{1}{4\Omega_1^2}\mathrm{H}_3^2-\frac{1}{2\Omega_1}\mathrm{G}_1\mathrm{H}_3\sin\Omega_1\tau)$$
$$= -[\frac{1}{4}(\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2)^2((\frac{1}{\Omega_1}\mathrm{H}_3\sin\Omega_1\tau - G_1)^2 - \frac{1}{4\Omega_1^2}\mathrm{G}_3^2)] \leq 0$$

Implies $(\frac{1}{\Omega_1}\mathrm{H}_3\sin\Omega_1\tau - G_1) \geq \frac{1}{2\Omega_1}\mathrm{G}_3.$

So $r_4 = 0$ implies $\frac{1}{4}\mathrm{H}_1^2+\sigma_2^2 = 0$ or $\frac{1}{\Omega_1}\mathrm{H}_3\sin\Omega_1\tau - G_1 = \pm\frac{1}{2\Omega_1}G_3$, consequently,

$$A = \frac{1}{\Omega_1}H_3\cos\Omega_1\tau$$

Substituting into r_3, we have $r_3 = 0$, so zero is not simple.

(i) Double zero and two negative eigenvalues

Taking $r_1 = 0.4,\ r_2 = 0.04,\ r_3 = r_4 = 0,$ then Equation (48) has a double zero and two negative eigenvalues, $\lambda_{1,2} = 0,\ \lambda_{3,4} = -0.2$. One choice of the parameters that satisfy these conditions is: $\Omega_1 = 2.6,\ H_1 = 0.3,\ \mathrm{H}_3 = 0.2, G_1 = 0.1,$ $G_3 = 1.67,\ \tau = 1.3,\ \sigma_1 = 0.1,$ $\sigma_2 = 0.1.$

(ii) Double zero and a pair of purely imaginary eigenvalues

Taking the following parameter values: $\Omega_1 = 2.6,\ H_1 = 0.037,\ \mathrm{H}_3 = 0.8, G_1 = 0.01,$ $G_3 = 0.8,\ \tau = 1.3,\ \sigma_1 = 2,\ \sigma_2 = 0.1,$ it yields that $r_1 = r_3 = 0$, $r_2 = 1$ in Equation (49) has a double zero and two imaginary eigenvalues $\lambda_{1,2} = 0,\ \lambda_{3,4} = \pm i$.

For the solution ($a \neq 0$ and $b = 0$), we get:

$$p_1' = (-\frac{1}{2}G_1 + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau)p_1 + (\frac{1}{4\Omega_1}G_3 - \frac{\sigma_1}{2} + \frac{1}{2\Omega_1}H_3\cos\Omega_1\tau - \frac{1}{2\Omega_1}H_3)q_1 \quad (51)$$

$$q_1' = (\frac{1}{2}\sigma_1 + \frac{1}{4\Omega_1}G_3 - \frac{1}{2\Omega_1}H_3\cos\Omega_1\tau + \frac{1}{2\Omega_1}H_3)p_1 + (-\frac{1}{2}G_1 + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau)q_1 \quad (52)$$

The stability of the linear solution is obtained from the zero characteristic equation

$$\begin{vmatrix} -\frac{1}{2}G_1 + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau - \lambda & \frac{1}{4\Omega_1}G_3 - \frac{\sigma_1}{2} + \frac{1}{2\Omega_1}H_3\cos\Omega_1\tau - \frac{1}{2\Omega_1}H_3 \\ \frac{1}{2}\sigma_1 + \frac{1}{4\Omega_1}G_3 - \frac{1}{2\Omega_1}H_3\cos\Omega_1\tau + \frac{1}{2\Omega_1}H_3 & -\frac{1}{2}G_1 + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau - \lambda \end{vmatrix} = 0 \quad (53)$$

hence $\lambda^2 + r_5\lambda + r_6 = 0,$

where $r_5 = G_1 - \frac{1}{\Omega_1}H_3\sin\Omega_1\tau$,

$$r_6 = \frac{1}{4}G_1^2 - \frac{1}{2\Omega_1}G_1H_3\sin\Omega_1\tau + \frac{1}{2\Omega_1^2}H_3^2 - \frac{1}{16\Omega_1^2}G_3^2 + \frac{1}{4}\sigma_1^2 + \frac{1}{2\Omega_1}\sigma_1H_3(1-\cos\Omega_1\tau) - \frac{1}{2\Omega_1^2}H_3^2\cos\Omega_1\tau.$$

Then we get $\lambda_{1,2} = \frac{1}{2}(-r_5 \pm \sqrt{r_5^2 - 4r_6})$

The linear solution is stable in this case if and only if $r_5 > 0$, and otherwise it is unstable.

Non-linear solution

To determine the stability of the fixed points, one lets

$$a = a_{10} + a_{11},\ b = b_{10} + b_{11},\ \varphi_m = \varphi_{m0} + \varphi_{m1}, (m = 1,2), \quad (54)$$

Where a_{10}, b_{10} and φ_{m0} are the solutions of Equations (36) to (39) and $a_{11}, b_{11}, \varphi_{m1}$ are perturbations which are assumed to be small compared to a_{10}, b_{10} and φ_{m0}. Substituting Equation (54) into Equations (32) to (35), using Equations (36) to (39) and keeping only the linear terms in $a_{11}, b_{11}, \varphi_{m1}$ we obtain:

For the solution ($a \neq 0$ and $b \neq 0$), we get:

$$\dot{a_{11}} = (-\frac{1}{2}\mathrm{G}_1 + \frac{1}{4\Omega_1}G_3 \sin\varphi_{10} + \frac{1}{2\Omega_1}H_3 \sin\Omega_1\tau)a_{11} - (\frac{1}{\Omega_1}\mathrm{G}_2\Omega_2^2\,\mathrm{b}_{10}\sin\varphi_{20})\mathrm{b}_{11}$$
$$+ (\frac{1}{4\Omega_1}G_3 a_{10}\cos\varphi_{10})\varphi_{11} - (\frac{1}{2\Omega_1}G_2\Omega_2^2\,\mathrm{b}_{10}^2\cos\varphi_{20})\varphi_{21} \quad (55)$$

$$\dot{\varphi_{11}} = (\frac{\sigma_1}{a_{10}} + \frac{1}{2\Omega_1 a_{10}}\mathrm{G}_3\cos\varphi_{10} - \frac{1}{\Omega_1 a_{10}}H_3\cos\Omega_1\tau)a_{11} + (\frac{2}{\Omega_1 a_{10}}\mathrm{b}_{10}G_2\Omega_2^2\cos\varphi_{20})\mathrm{b}_{11}$$
$$+ (\frac{1}{2\Omega_1}\mathrm{G}_3\sin\varphi_{10})\varphi_{11} + (\frac{1}{\Omega_1 a_{10}}G_2\Omega_2^2\,\mathrm{b}_{10}^2\sin\varphi_{20})\varphi_{21} \quad (56)$$

$$\dot{b_{11}} = (\frac{1}{4\Omega_2}\mathrm{H}_2\Omega_1^2\,\mathrm{b}_{10}\sin\varphi_{20})a_{11} + (-\frac{1}{2}H_1 + \frac{1}{4\Omega_2}\mathrm{H}_2\Omega_1^2 a_{10}\sin\varphi_{20})\mathrm{b}_{11}$$
$$+ (\frac{1}{4\Omega_2}\mathrm{H}_2\Omega_1^2 a_{10}\,\mathrm{b}_{10}\cos\varphi_{20})\varphi_{21} \quad (57)$$

$$\dot{\varphi_{21}} = (\frac{1}{2\Omega_2}\mathrm{H}_2\Omega_1^2\cos\varphi_{20} - \frac{\sigma_1}{2a_{10}} - \frac{1}{4\Omega_1 a_{10}}\mathrm{G}_3\cos\varphi_{10} + \frac{1}{2\Omega_1 a_{10}}H_3\cos\Omega_1\tau)a_{11}$$
$$+ (\frac{1}{b_{10}}(\frac{\sigma_1}{2} - \sigma_2) + \frac{1}{2\Omega_2 b_{10}}a_{10}H_2\Omega_1^2\cos\varphi_{20} - \frac{1}{\Omega_1 a_{10}}\mathrm{b}_{10}G_2\Omega_2^2\cos\varphi_{20})\mathrm{b}_{11}$$
$$- (\frac{1}{4\Omega_1}\mathrm{G}_3\sin\varphi_{10})\varphi_{11} + ((\frac{1}{2\Omega_2}a_{10}H_2\Omega_1^2 - \frac{1}{2\Omega_1 a_{10}}G_2\Omega_2^2\,\mathrm{b}_{10}^2)\sin\varphi_{20})\varphi_{21} \quad (58)$$

The stability of a particular fixed point with respect to perturbations proportional to $\exp(\lambda t)$ depends on the real parts of the roots of the matrix. Thus, a fixed point given by Equations (55) to (58) is asymptotically stable if and only if the real parts of all roots of the matrix are negative.

For the solution ($a \neq 0$ and $b = 0$), we get:

$$\dot{a_{11}} = (-\frac{1}{2}\mathrm{G}_1 + \frac{1}{4\Omega_1}G_3\sin\varphi_{10} + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau)a_{11} + (\frac{1}{4\Omega_1}G_3 a_{10}\cos\varphi_{10})\varphi_{11} \quad (59)$$

$$\dot{\varphi_{11}} = (\frac{\sigma_1}{a_{10}} + \frac{1}{2\Omega_1 a_{10}}\mathrm{G}_3\cos\varphi_{10} - \frac{1}{\Omega_1 a_{10}}H_3\cos\Omega_1\tau)a_{11} + (\frac{1}{2\Omega_1}\mathrm{G}_3\sin\varphi_{10})\varphi_{11} \quad (60)$$

The stability of a given fixed point to a disturbance proportional to $\exp(\lambda t)$ is determined by the roots of:

$$\begin{vmatrix} -\frac{1}{2}\mathrm{G}_1 + \frac{1}{4\Omega_1}G_3\sin\varphi_{10} + \frac{1}{2\Omega_1}H_3\sin\Omega_1\tau - \lambda & \frac{1}{4\Omega_1}G_3 a_{10}\cos\varphi_{10} \\ \frac{\sigma_1}{a_{10}} + \frac{1}{2\Omega_1 a_{10}}\mathrm{G}_3\cos\varphi_{10} - \frac{1}{\Omega_1 a_{10}}H_3\cos\Omega_1\tau & \frac{1}{2\Omega_1}\mathrm{G}_3\sin\varphi_{10} - \lambda \end{vmatrix} = 0 \quad (61)$$

Consequently, a non-trivial solution is stable if and only if the real parts of both eigenvalues of the coefficient matrix Equation (61) are less than zero.

NUMERICAL RESULTS

Runge-kutta fourth order method has been conducted to determine the numerical solution of the given system. Figure 2 illustrates the response and phase-plane for the non- resonant system at some practical values of the equations parameters without time delay control.

Resonance cases

we see that the amplitude increasing at the resonance cases and the worst case is the simultaneous resonance case when $\Omega = 2\Omega_1,\ \Omega_1 = 2\Omega_2,$ which the amplitudes are increased to about 175% compared with the basic case shown in Figure 3, which means that the system need s to reduced the amplitude of vibration or controlled.

Figure 4 shows the effects of time delayed, from this figure we can see that the delayed is effect at the regions $1.1 \le \tau \le 2.2$, $3.5 \le \tau \le 4.5$ and $5.9 \le \tau \le 6.9$, otherwise not affect. Figure 5, illustrates system at the worst case is the simultaneous resonance case when $\Omega = 2\Omega_1,\ \Omega_1 = 2\Omega_2,$ with time delay control the amplitude of vibrations is very minimum , which means that the time delay control is very effective.

Effect of parameters

The amplitude of the system is monotonic decreasing function of the coefficient gain G_1 more increasing of G_1 leads to saturation phenomena as shown in Figure 6a. The amplitude is a monotonic increasing function of the excitation amplitude of the coefficient G_3. But more increasing of coefficient G_3 leads to very high amplitude and the system becomes unstable as shown in Figure 6b

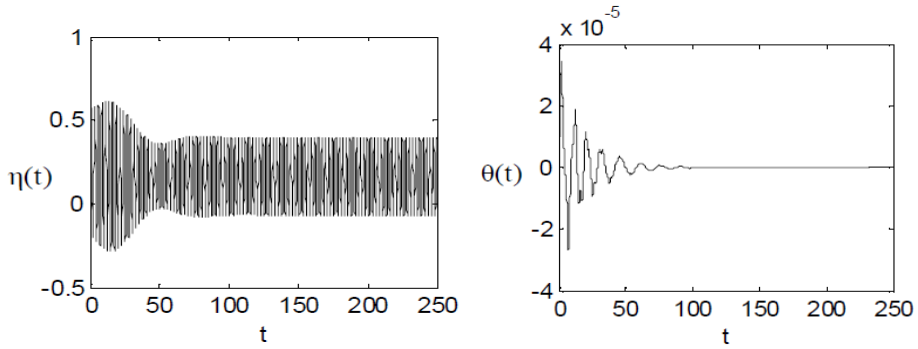

Figure 2. Basic case without control (Ω_1=2.4, Ω=2.5, Ω_2=0.3, H_3=0, G_1=0.1, G_2=0.9, G_3=0.8, H_1=0.1, H_2=0.7).

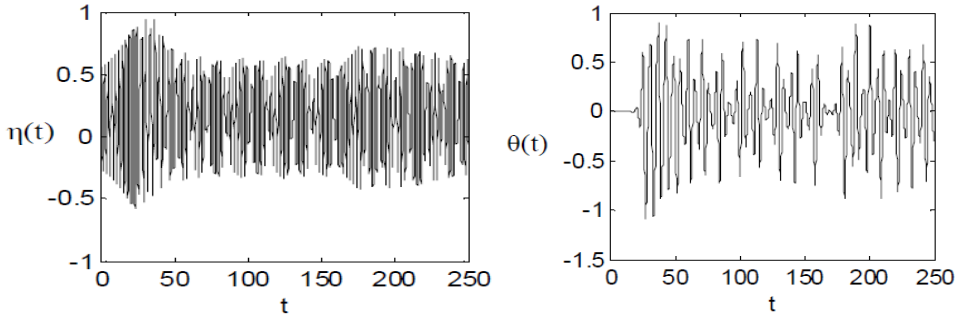

Figure 3. Resonance case without control (Ω_1=2.6, Ω_2=1.3, Ω=5.2, H_3=0, G_1=0.1, G_2=0.9, G_3=0.8, H_1=0.1, H_2=0.7).

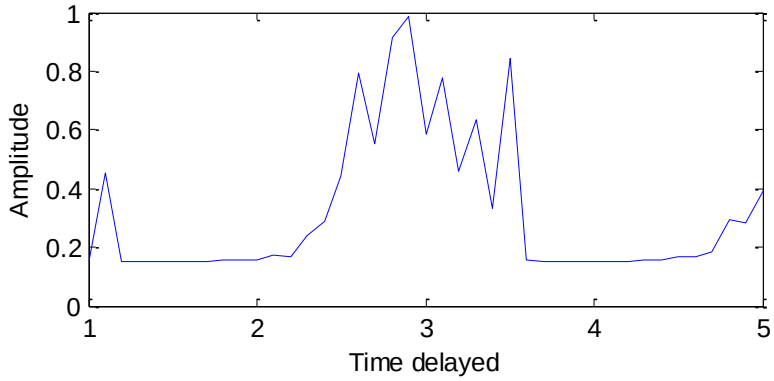

Figure 4. Effects of time delayed (τ) on the system.

Response curves

The frequency response Equations (40) and (41) are nonlinear algebraic equations of a, b. These equations are solved numerically as shown in Figures 7 and 8. From Case 1 where $a \neq 0$, $b \neq 0$: Figure 7, shown that the steady state amplitudes of the system are monotonic decreasing functions in Ω_1, G_1. But the steady state amplitudes of the system are monotonic increasing functions in G_2, G_3, H_3.

Comparison between numerical solution and approximation solution

Now, we get good agreement of the approximate solution obtained from frequency response equation and the

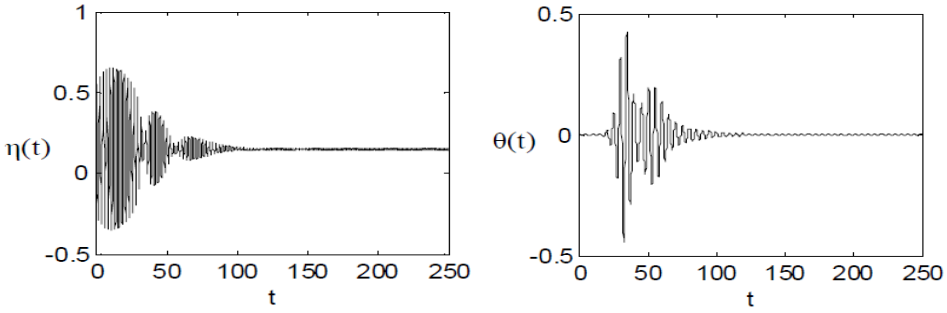

Figure 5. Resonance case with control (Ω_1=2.6, Ω=5.2, Ω_2=1.3, H_3=0.2, G_1=0.1, G_2=0.9, G_3=0.8, H_1=0.1, H_2=0.7, τ=1.3).

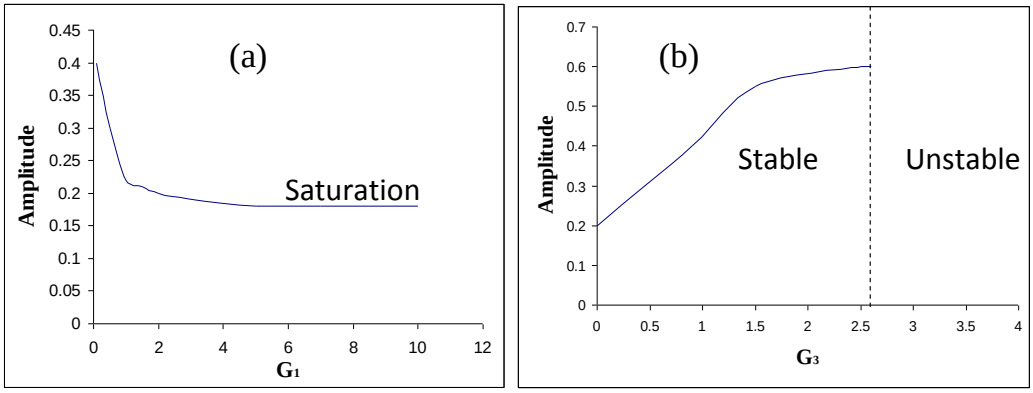

Figure 6. Effect of parameters.

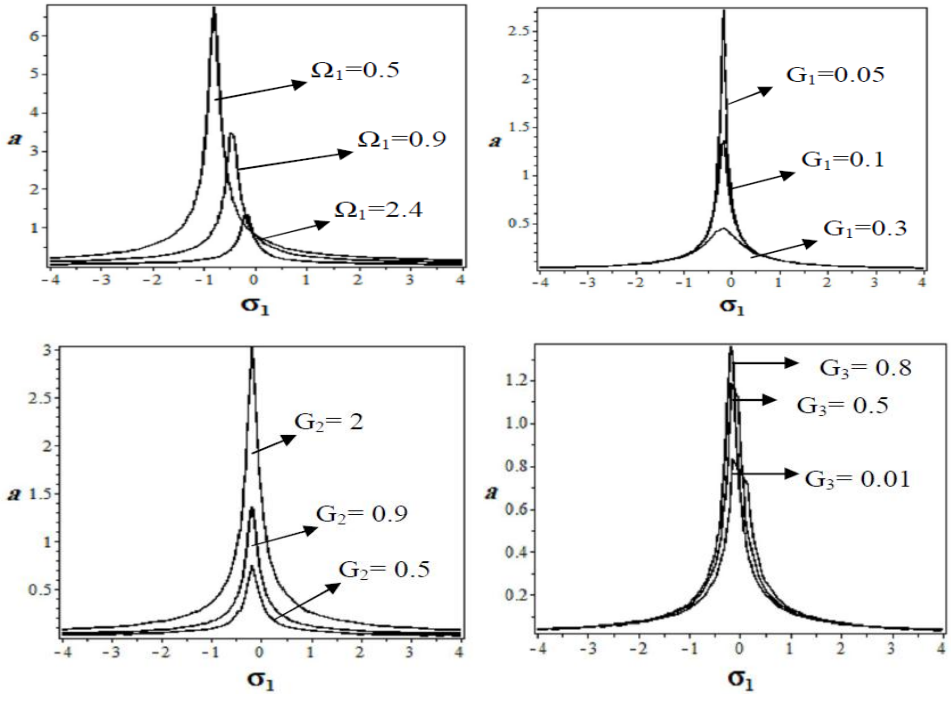

Figure 7. Frequency response curves ($a \neq 0$ *and* $b \neq 0$).

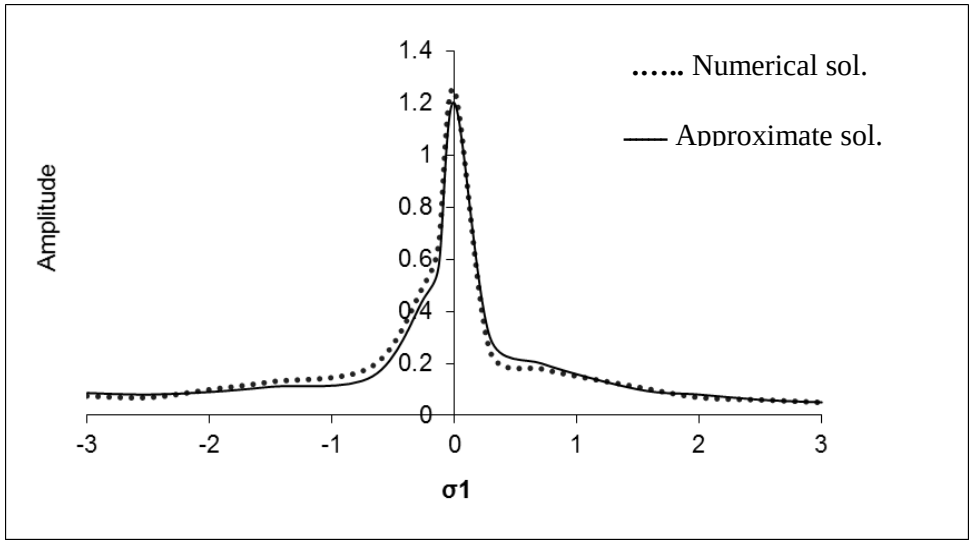

Figure 8. Comparison between numerical solution and approximation solution.

numerical solution obtained by Runge Kutta forth order method as shown in Figure 8.

CONCLUSIONS

The response and stability of the system of coupled non-linear differential equations representing the non-linear dynamical two-degree-of-freedom the system is studied. The time delayed feedback control is applied to suppress the vibration of the system. The equation of motion and the perturbation analysis and the stability of the equilibrium solutions are given. The analytical solutions and numerical simulations of the delayed feedback control are presented. The investigation includes the solutions applying both the perturbation technique, and Runge-Kutta numerical method. Also a comparison with similar published work is reported. Here, the main conclusions of the system are reported briefly.

(1) The worst resonance case is the simultaneous resonance case when $\Omega = 2\Omega_1$, $\Omega_1 = 2\Omega_2$, which the amplitudes are increased to about 175% compared with the basic case.

(2) The most effective regions of the time delayed is effect are $1.1 \le \tau \le 2.2$, $3.5 \le \tau \le 4.5$ and $5.9 \le \tau \le 6.9$, otherwise not effect.

(3) The time delayed is very powerful technique to reduce the vibration of the system at the simultaneous resonance case.

(4) The steady-state amplitude is monotonic decreasing functions in the gain G_1, and monotonic increasing of the excitation amplitude G_3.pectively.

(5) The approximate solution is good agreement with numerical solution.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Evaluation of agro-ecological approach to soil quality assessment for sustainable land use and management systems

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Long-term cultivation of crops has been found to cause soil physico-chemical and biological quality (SQ) degradation for sustainable agricultural production practices in Akwanga, Nigeria. Understanding the dynamics of SQ change under different land use types is desired. The present study was carried out to assess soil physico-chemical indicators (based on land evaluation approach) and biological SQ (based on agro-ecological approach) for sustainability of cereal, arable and plantation land uses and management systems. Quantitative and qualitative indicators were defined based on chronosequence of soils under different land use types: 3-month cereal cropping of Rice); 7-month root cropping (yam/cassava/vegetable intercrop); 10-year plantation (orange/pineapple orchard); and >22-year oil palm plantation. Their respective management practice is Tillage + NPK fertilization; Tillage + NPK fertilizers + Organic manure; No tillage + mulch; and no tillage + Farm yard manure + Legume cover as live mulch. Each age class was replicated at least three times and their sensitivity to change was sought. The statistical mean values of the bio-physical and chemical properties of soil quality indicators (SQI) under the various types of land use (LUT) show that the most sensitive soil quality indicators ($P \leq 0.001$) were soil pH, total organic C, available phosphorus (P), CEC, bulk density, total porosity, and PWAC, and earthworm population. Moderately sensitive indicators ($0.001 < P \leq 0.01$) include total N, P and K, and exchangeable K. Weaker indicators of SQ ($0.01 < P \leq 0.05$) include percentage BS. Soil texture and clay/silt ratio were of no value as soil quality indicators (SQI) for these soils. SQI improvements were related to their management practices; hence LUT4 had the best SQ, followed by LUT3. The worst management was that in LUT1. Qualitative assessments based on farmers' perception of SQ showed the following order of importance for their cropping systems: Soil organic matter, fertility, topsoil thickness, soil structure, moisture retention, earthworm abundance, compaction, soil erosion, and the incidence of weeds. Farmer observations of SQ changes were generally in good agreement with the quantitative assessments. To ensure adoption of improved management practices for more sustainable production system, qualitative soil quality information obtained from on-farm surveys should be used to supplement the quantitative data obtained through soil analyses. These would serve as effective diagnostic tools for evaluating soil quality for long-term sustainability of crop productivity

Key words: Soil quality indicators, land evaluation, agro-ecological approach, guinea savanna, Nigeria.

INTRODUCTION

In Guinea savanna agro-ecology of Nigeria, arable, cereal and perennial cash crop production are dominant land uses. Their production system is undergoing major changes in response to population pressure upon land resources to meet their food and fibre demand. As a consequence, there has been an increase in both land use intensity and soil degradation (Ezeaku and Salau, 2005).

Intensive land use causes important changes in soil physico-chemical and biological characteristics, and can rapidly diminish soil quality and soil fertility. This follows Amana et al. (2012) report that ecologically sensitive components of tropical soils are not able to buffer effect of intensive agricultural practices. Thus, severe deterioration of soil quality (SQ) may lead to a permanent degradation of land productivity.

Initially planted crops remain productive for long periods but yields tend to decline in later years, especially plantation crops. This drop in productivity is traditionally associated to natural ageing of the plants (Do, 1980), while low yield from other land uses may reflect a loss in soil quality (SQ) due to the type of intensive land use involved in the production. Moreover, because crop growth and productivity are a reflection of SQ (Penning de Vries et al., 1995a), any degradation of the soil can be expected to adversely affect the stability of soil system in the tropics (Ezeaku, 2013). The spatial distribution of any soil has a marked influence on its agricultural productivity (Obasi et al., 2011), while the extent and impact of soil degradation can also lead to the reduction of biological and economic productivity potentials of rain-fed or irrigated croplands, pasture and forested land, including social and political instability (Adaikwu et al., 2012; Ezeaku and Iwuanyanwu, 2013).

The foregoing revives the issue of sustainability; hence it was deemed that an evaluation of SQ changes could enhance the sustainability of crop production in guinea savanna agro-ecology of Nigeria.

Awareness that soil is vital to both production of food and fibre and global ecosystem functions generated interest in the quality and health of soil for environmental sustainability (Bouma, 2002). Hence, during the last decade a soil quality concept emerged, necessitating several SQ definitions and quantifications (Piarce and Larson, 1993; Doran and Parkin, 1994; Karlen et al., 1997; Mausbach et al., 1998; Nortcliff, 2002). The summary of these authors' definitions is that soil quality is "*the capacity of a specific kind of living soil to function, within ecosystem boundaries to sustain biological productivity (plant and animal), maintain environmental (air and water) quality, and support/promote plant and animal health and habitation".*

Some bio-physicochemical indicators that determine a soil's quality to function have been identified (USDA, 1993) and include: Soil depth, water-holding capacity, bulk density, nutrient availability, potential capacity, organic matter, microbial biomass, carbon and nitrogen content, soil structure, water infiltration, and crop yield. These determine soil's two distinct but interconnected parts (Mausbach et al., 1998): i) inherent quality (that is, innate properties) of soils as determined by the factors of soil formation-climate, topography, vegetation, parent material, and time. An example is water holding capacity that determines inherent quality for storing water; and ii), dynamic quality, which results from the changing nature (health or condition) of soil properties as influenced by human use and management decisions. Management practices and uses of the land either result in a net positive (e.g. increased organic matter contents of soils under irrigation) or negative (e.g. compaction from tillage or acidification from fertilizer application) impact on the health of the soil (Mausbach et al., 1998). This dynamic aspect of soil quality is the focal point of the concern for assessing and maintaining healthy soil resources, a point of emphasis in this study.

As soil quality integrates the biological, chemical, and physical components and processes of the soil interconnected with its surroundings in the landscape (Arshad and Coen, 1992), much remains to be known concerning the complex relationships between specific soil property measurements and overall soil quality (Gomez et al., 1996). Therefore, a methodology for assessing and monitoring soil quality whether the setting is a research plot, a field, watershed, or earth from space (global) is necessary (Seybold et al., 1998).

Several minimum data sets of indicators have been proposed (Ezeaku, 2013; Raji, 2011; Doran and Jones, 1996; Gregorich et al., 1994; Larson and Pierce, 1994). An example minimum data set is presented in Table 1 (Seybold et al., 1998). However, for indicators to be useful in assessing soil quality, a standard or reference condition must be established as a baseline from which to assess the current state of soil quality (Karlen et al., 1997). This determines whether soil quality, from environmental viewpoint, is improving, stable, or declining with changes in land use (Sanchez-Maranon et al., 2002). If the change in a soil quality indicator is positive and more is of better quality, then the soil can be regarded as improving or aggrading in quality. Conversely if the trend line is negative, then soil quality is degrading. Therefore, use of the reference condition in conjunction with trend analysis to monitor change in soil quality could be better.

Qualitative (descriptive) and quantitative (analytical) approaches have been most commonly used for

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monitoring and assessing soil quality changes (Ezeaku and Iwuanyanwu, 2013; de la Rosa et al., 2009; Harris et al., 1996). Input variables or diagnostic indicators expressed in the traditional land evaluation (quantitative and qualitative approaches) for soil quality assessment include land characteristics such as soil physic-chemical properties, climate and crop/management factors as well as soil degradation processes (Tables 1 to 3) (Seybold et al., 1998; de la Rosa et al., 2004; Ezeaku, 2005), but these parameters are mainly capable of indicating different end-point values in a soil retrospective (Filip, 2002). Therefore, it appears appropriate to develop soil-quality assessment that incorporates biological soil indicators in order to assess the total sustainability of soil (agro-ecology) functions under different uses.

Akwanga located in guinea savanna agro-ecology of Nigeria has various natural environments containing native and managed soils with different land uses and management systems. Therefore, the study was carried out to assess soil quality indicators based on land evaluation approach (physical/chemical properties) and to explore agro-ecological approach (biological property) for sustainability of land use and management systems in the study area.

MATERIALS AND METHODS

Study site description

The study was conducted for two years (2005-2006 cropping seasons) in Akwanga location (6° 15'N and 9° 30' E and 11° 00' E, with altitude about 600 m above sea level) at Nasarawa state belonging to guinea savanna agroecology of Nigeria. It is an agricultural area generally characterized by gentle, undulating plains and upland-inland continuum. The general climate is humid tropical, having distinct rainy with clear and dry seasons. The mean temperature ranges between 23.5 and 30.9°C, while mean annual rainfall ranges between 1270 and 1530 mm with a 3 to 4 month dry season. The dominant land uses are plantation, cereal and arable cropping systems (Ezeaku and Salu, 2005; Amana et al., 2012).

The study was based on chronosequence approach which represents an ecological time series of soil where the differences in age or time of land use are selected but not differences in environmental conditions (Dyck and Cole, 1994). This method is often used to define the degree of soil degradation or improvement by comparing soil properties under the same or different land use patterns but having different land use periods (Karlen et al., 1994). Based on such approach and for the purpose of this study, field sites were randomly selected based on dominant land uses, age/time and management for soil sampling, that is, 3-month (cereal cropping: Rice); 7-month (root cropping: Yam/Cassava/Vegetable intercrop) as short-term crop cultivation; 10-year (plantation: Orange/Pineapple orchard); and >22-year (plantation: Oil palm) as long-term crop production. Their respective management is Tillage + NPK fertilization; Tillage + NPK fertilizers + Organic manure; No tillage + mulch; and No tillage + farm yard manure + Legume cover as live mulch. Each age class was replicated at least three times.

Due to the beneficial effects of permanent vegetation cover on soil physical, chemical, and biological properties (Burger et al., 1998), natural vegetation areas adjacent to the various land use types was expected to provide optimal values (reference conditions) to compare the current state of soil characteristics of the sites with various land uses. If the change in a SQI is positive, then the SQ is improving, but if the trend line is negative, then the SQ is degrading.

Quantitative approach (soil analysis)

Soil sampling procedures

Physical analyses (particle size distribution, plant available water-holding capacity- Plant available water-holding capacity (PAWC), bulk density) and total porosity were conducted, respectively, using composite and undisturbed samples (n = 24) collected using cylindrical cores (at the 0-20 cm depth only) from three grids (7 × 10 m) within each field.

Soil samples analyzed for chemical were collected with auger samplers at two depths (0-20 cm surface soil; 20-40 cm subsurface soil). Each depth increment was composited and analyzed separately for the following soil properties: pH; Organic carbon; total N; exchangeable cations - Ca, Mg, Na, K; available P; total P and K with CEC and Base saturation calculated. Total soil P and K were analyzed to understand their accumulation in soils. Earthworm populations, representing biological indicators, were monitored monthly throughout the rainy season (June-October, 2006). Five core (15 cm diameter) soil samples were randomly collected from each land use type soil (25 × 25 × 20 cm, even though earthworms have been shown to be primarily in the surface 15 cm of soil profile (Pankhurst, 1997) to determine the earthworm population.

Laboratory methods

The analytical characteristics of the soil samples were determined in the following manner. A particle size analysis was determined by Gee and Bauder (1986) method. Soil pH was obtained in 1:2.5 soil/water extract of the composite samples according to McLean (1982) method. Organic carbon (OC) was determined by the potassium dichromate method (Nelson and Sommers, 1982); Organic matter was obtained by multiplication of OC with a factor 1.72. Exchangeable cations (Ca, Mg, Na and K) were estimated using 1M NH_4 OAC extractant method (Thomas, 1982) where Ca and Mg were obtained on an Atomic Absorption Spectrometer; Na and K by flame photometer; Cation exchange capacity (CEC) was obtained as a summation of exchangeable bases and acidity (Rhoades, 1982a). Total N was determined by Macro-Kjeldhal method (Bremmer and Mulvaney, 1982). Available phosphorus (P) was obtained by Bray I extractant (Olsen and Sommers, 1982) method. Total soil P and K were extracted using H_2SO_4-H_2O_2 digestion (Thomas et al., 1967). Phosphate in the digests was measured colorimetrically using a Technicon autoanalyzer; K in the extracts was determined using absorption spectrometry (AES). Bulk density was estimated by core method described by Blake and Hartge (1986). Plant available water-holding capacity (PAWC) was calculated as the difference between field capacity and the permanent wilting point–determined using a pressure chamber apparatus (Anderson and Ingram, 1993) and the values expressed on percent volumetric basis. Total porosity was estimated from the particle and bulk density and value expressed on percentage basis. For the earthworm populations' determination the core samples were passed through a 10mm sieve to hand-sort the earthworms. Earthworm data represents the cumulative number of each monthly sampling.

Qualitative approach (farmer interviews)

A survey was conducted at the same time and the same place where soil samples were taken in order to explore indigenous

Table 1. Proposed minimum data set of physical, chemical and biological indicators for screening the condition, quality, and health of soil.

Indicators of soil condition	Relationship to soil condition and function; Rationale as a priority measurement.
Physical	
Texture	Retention and transport of water and chemicals; Modeling use, soil erosion and variability estimate.
Depth of soil, topsoil, and rooting	Estimate of productivity potential and erosion; Normalizes landscape and geographic variability.
Infiltration and bulk density	Potential for leaching, productivity, and erosivity; SDB needed to adjust analyses to volumetric basis.
Water holding capacity	Related to water retention, transport and erosivity; Available H_2O, calculate from SDB, texture and OM.
Chemical	
Soil organic matter (SOM)	Defines soil fertility, stability and erosion extent; use in process models and for site normalization.
Soil pH	Defines biological and chemical activity thresholds; Essential for process modeling.
Electrical conductivity	Defines plants and microbial activity thresholds; Presently lacking in most process model.
Extractable N, P and K	Plant available nutrients and potential for N loss; Productivity and environmental quality indicators.
Biological	
Microbial biomass C and N	Microbial catalytic potential and repository for C and N; Modeling: early warning of management effects on OM.
Potentially mineralizing N	Soil productivity and N supplying potential; process modeling (surrogate indicator of biomass.
Soil respiration, water content and temperature	Microbial activity measure (in some plants), Process modeling; estimate of biomass activity.

Source: Seybold et al. (1998)

Table 2. List of land productivity and degradation related issues, the input land characteristics required and their modeling procedures.

Input land characteristics required	Issues evaluated			
	Soil	Climate	Crop/management	Modeling procedure
Land productivity-related				
General land capability	+	+	_	Qualitative
Agricultural soil suitability	+	-	-	Qualitative
Forestry land suitability	+	+	-	Qualitative
Natural soil fertility	+	-	-	Qualitative
Soil productivity	+	-	-	Statistical
Bioclimatic deficiency	+	+	+	Parametric
Land degradation related				
General soil contamination	+	+	+	Expert system
Specific soil contamination	+	+	+	Expert system
Water and wind erosion	+	+	+	Expert system
Soil plasticity/Workability	+	-	-	Statistical system
Subsoil compaction	+	+	+	Statistical system
Erosion/ impact/mitigation	+	+	+	Expert system/neural network

+, Required; -, not required. Source: de la Rosa et al. (2004).

knowledge of the farmers. Farmers approach is descriptive using words as descriptors, and hence, is inherently subjective. A questionnaire was developed based on the soil quality survey proposed by Garlynd et al. (1994). The questionnaire was used as an interview guide, in which the questions were structured in a way that was understood easily by the farmers. This is akin to the soil health card proposed by USDA Soil Quality Institute (Romig et al., 1995). The questionnaire guide was pre-tested and corrected to be sure the research objectives were satisfied.

The survey included 22 farmers chosen at random from the

community. Only heads of household who have at least 20 years working experiences in farms were interviewed. These heads of household had some types of formal education, with approximately 23% having completed a high school level education, 42% at a secondary school level, and only 35% at a primary school level. Household heads were selected and interviewed as representative of the four main cropping systems sampled.

Statistics

Differences between the different land use practices based on the several soil biophysical and chemical properties were determined (Hoshmand, 1994). Significant differences between means were identified using sensitivity analysis (t-test). For the purpose of this study, a given soil property was considered to be a sensitive indicator of soil quality if the probability of a greater F-value (P>F) was ≤ 0.05. Moreover, the smaller the probability value, the greater the sensitivity of the indicator variable. Conversely, a given soil property was considered to be a poor indicator of soil quality if the probability of a greater F-value was >0.05. The use of t-test was to verify if there were statistically significant differences. Changes in these indicators can be used to determine whether soil quality is improving, stable or declining with changes in land use.

RESULTS AND DISCUSSION

Quantitative soil quality indicators (Sensitivity analysis)

Potential soil quality indicators assessed in this study included a variety of soil physical, chemical, and biological properties. To be useful as an indicator of soil quality, variations in soil properties associated with management practice must be distinguishable from those associated with natural soil variability (Burger and Kelting, 1998).

The statistical mean values of the bio-physical and chemical properties of soil quality indicators (SQI) under the various types of land use (LUT) are presented in Table 4. The results show that the most sensitive soil quality indicators ($P \leq 0.001$) were soil pH, total organic C, available phosphorus (P), CEC, bulk density, total porosity, and PWAC, and earthworm population. Moderately sensitive indicators ($0.001 < P \leq 0.01$) include total N, P and K, and exchangeable K. Weaker indicators of soil quality ($0.01 < P \leq 0.05$) include percentage BS. Soil properties such as soil texture and clay/silt ratio exhibited little change with cultivation history and, consequently, were of no value as soil quality indicators for these soils.

Assessment of dynamic SQI under different land uses

To fully assess the impact of cultivation on soil quality, it is necessary to have a baseline against which cultivation induced differences can be measured (Burger and Kelting, 1998). The reference condition for this study was native vegetation nearby the crop fields. Reference was also made to the critical values of soil properties established for the tropics (Ojanuga and Awojuola, 1981) and as well used in discussing data in Table 4 and contrasted LUT values of the reference conditions (natural vegetation fallow soils) as shown in Table 5. The statistical results showing the direction of change in population mean are presented in Table 6 as > (increase), < (decrease) and = (no change or static).

The result (Table 4) shows that texturally the soils are generally uniform in clay content and the silt/clay ratio is less than unity, suggesting high weatherability of the soils and pedogenesis under land uses (Nwaka and Kwari, 2000).

Bulk density of 3-month LUT (1.33 mgm^{-3}) was higher than that of 7-month (1.30 mgm^{-3}) and this may be related to the physiographic position of the LUT soils. Upland Rice/Maize (3-month) LUT was cultivated in slightly lower land than Yam/Cassava (7-month) LUT located in the upper landscape. It is expected that colluviation and seasonal flooding of soils, resulting to continued wetting and drying of soils (Areola, 1982), may have contributed to the increased bulk density. Caron et al. (1992) and Swartz et al. (2003) reported increases in bulk density due to decreases in aggregate stability leading to collapse of soil pores (decreased macro-porosity) and production of finer particles and macro-aggregates. Similarly, high soil bulk density observed in 7-month LUT relative to reference condition (Table 4) may be associated to poor vegetal cover, soil surface crusting and compaction by raindrop impact.

High percentage porosity observed across the LUTs may be due to decreased bulk density (Table 4) and could be the cause of increased availability of water (PAWC) relative to reference conditions. Thus increase in bulk density of the reference fallow condition may be due to compaction that could inhibit water conductance and availability, oxygen movement to the root zone and especially; the erosive vulnerability of macro-aggregates (Karlen et al., 1997). These are in further agreement with the report that structural decline due to compaction, typical of some agricultural systems; specifically affect the transmission and drainage pores (Caron et al., 1992).

Adaikwu et al. (2012) reported that a typical characteristic of savanna soils is that pH of the soil in water is predominantly moderate to slight acid condition. The mean soil pH values obtained across the LUTs ranges from 6.2 to 6.8 (Table 4). These values could be considered reasonably well for plant growth and development in the area. The pH values obtained accords the range (6.2 to 6.5) reported for soils in southern guinea savanna of Nigeria (Adaikwu et al., 2012; Amana et al., 2012; Akinrinde and Obigbesan, 2000). The increased pH of the soils may be associated to incorporated vegetation biomass that has the capacity to retain and release enough base forming cations. Value of 17.4 gkg^{-1} organic-C content (OC) is suggested as critical limit level for the soils of northern Nigeria (Akinrinde and

Table 3. Main soil characteristics and qualities considered in land evaluation.

Grouping type	Soil physico-chemical parameters**
Visible attributes	Surface ponding of water, surface runoff, forms of rill, sheet or gully, exposure of subsoil, sub-soil compaction, retarded/poor growth.
Physical attributes	Soil texture, bulk density, porosity, aggregate strength and stability, soil crusting, soil compaction, water retention, drainage, hydraulic conductivity, infiltration rate, stoniness, soil depth.
Chemical attributes	Clay content, soil reaction, colour, organic matter content, carbonate content, base saturation, cation exchange capacity, sodium saturation, electrical conductivity, soil fertility status.
Land qualities*	
Land Productivity	Nutrient availability, water holding capacity/ availability, oxygen availability that is, water and air-filled pores), plant root penetration, plant- water- use efficiency, crop growth.
Land Degradation	Soil structure, cover protection, runoff, soil erodibility, sub-soil compaction, soil workability, leaching potential, toxic absorption and mobility, pesticide degradation.

Sources: de la Rosa et al. (2004)*; Ezeaku (2005)**.

Table 4. Soil quality data (mean and standard deviation) among the fallow and age of the various land use types (LUT).

Indicator	Fallow (n=6)	3-month (n=4)	7-month (n=4)	10-year (n=5)	>22-year(n=5)	Sig. level
Soil physical indicators						
Clay gkg^{-1}	22(8.3)	24(8.0)	22(6.9)	21(7.1)	23(7.6)	*
Si/Clay ratio	0.84	0.80	0.77	0.81	0.87	ns
Bd Mgm^{-1}	1.37(3.9)	1.33(3.1)	1.30(3.4)	1.28(2.7)	1.26(3.8)	***
Tp (%)	52.7(10.3)	54.3(10.9)	55.1(10.3)	57.9(9.1)	60.0(8.7)	***
PAWC(% V)	9.1(3.1)	9.7(3.4)	9.4(3.6)	10.5(3.4)	13.1(3.6)	***
Soil chemical indicators						
Soil pH (H_2O)	6.8(0.03)	6.2(0.04)	6.4(0.02)	6.5(0.03)	6.6(0.02)	***
Total C gkg^{-1}	10.8(0.6)	4.2(0.01)	2.9(0.03)	17.6(7.8)	21.0(9.2)	***
Total N gkg^{-1}	2.6(1.2)	0.9(0.1)	1.2(0.1)	2.4(0.6)	4.1(0.5)	**
Av.P $mgkg^{-1}$	24.0(5.2)	22.4(3.4)	19.0(3.2)	23.8(5.7)	24.5(5.3)	***
Exch.K $cmolkg^{-1}$	13.4(2.2)	11.3(3.1)	12.7(2.6)	13.3(1.9)	13.9(1.7)	ns
Total P $kgha^{-1}$	320(52)	262(67)	247(91)	338(76)	360(89)	**
Total K $kgha^{-1}$	263(56)	164(40)	179(24)	256(53)	262(58)	**
CEC $cmolkg^{-1}$	5.4(4.1)	5.3(2.8)	4.7(3.8)	8.6(2.5)	10.3(1.4)	*
BS (%)	96(22)	94(21)	92(22)	97(23)	96(23)	ns
Bio-indicators						
Ew count m^{-3}	10	2	3	7	9	***

[a] = significant at 0.05 (*), 0.01 (**) and 0.001(***) level of probability; ns = not significant; Cc = clay content; Si/Cl = silt/clay; Bd = bulk density; Tp(%) = total porosity percent; PAWC (%v) = plant available water capacity on volumetric percent; C = carbon; N = nitrogen; av.P = available phosphorus; Exch. K = exchangeable potassium; CEC = cation exchange capacity; BS(%) = base saturation percent; Ew = earthworm.

Obigbesan, 2000). The result (Table 4) shows that total OC contents of the 3-month (4.2 gkg^{-1}) and 7-month (2.9 gkg^{-1}) soils are below the critical limit; an indicative of very high biological degradation. Low soil organic matter content (SOM) may be due to crop uptake exacerbated by continuous cropping without adequate measures of nutrient replacement either through the use of inorganic fertilizer or other forms of soil conservation measures (Adaikwu et al., 2012). They also reported that low SOM is a process associated with the savanna soils, which could be due to high temperature that rapidly breakdown organic matter and inhibit nitrogen fixation by rhizo-bacteria. Asadu et al. (2004) associated low SOM with use of inappropriate farming practices, frequent changes in land uses (over cultivation) and erosion. These could result to decline in crop performances.

Table 5. Statistical level (P>F) for contrasts among land use type (LUT) study soils.

Indicator	Fallow Vs 3-month	3-month Vs 7 month	Fallow Vs 10-year	10-year Vs 22 year
Soil physical indicators				
Bd Mgm^{-1}	0.000	0.002	0.008	0.212
Tp (%)	0.001	0.001	0.003	0.241
PAWC(% V)	0.002	0.006	0.009	0.073
Soil chemical indicators				
Soil pH (H_2O)	0.001	0.056	0.001	0.051
Total C gkg^{-1}	0.000	0.004	0.010	0.143
Total N gkg^{-1}	0.001	0.001	0.008	0.092
Av.P $mgkg^{-1}$	0.002	0.002	0.000	0.006
Exch.K $cmolkg^{-1}$	0.000	0.001	0.003	0.042
Total P $kgha^{-1}$	0.000	0.000	0.002	0.000
Total K $kgha^{-1}$	0.000	0.012	0.000	0.038
CEC $cmolkg^{-1}$	0.002	0.000	0.009	0.027
Bio-indicators				
Ew count m^{-3}	0.000	0.000	0.001	0.003

Bd = bulk density; Tp (%) = total porosity percent; PAWC (%v) = plant available water capacity on volumetric percent; C = carbon; N = nitrogen; Ext. P = extractable phosphorus; Exch. K = exchangeable potassium; CEC = cation exchange capacity; BS(%) = base saturation percent; Ew = earthworm. Only dynamic soil properties were selected.

Table 6. Changes in soil quality indicators in response to crop cultivation.

Indicator	Fallow Vs 3-month	3-month Vs 7 month	Fallow Vs 10-year	10-year Vs 22 year
Soil physical indicators				
Bd Mgm^{-1}	<	<	<	<
Tp (%)	>	>	>	>
PAWC(% V)	>	<	>	>
Soil chemical indicators				
Soil pH (H_2O)	<	<	<	=
Total C gkg^{-1}	<	<	=	>
Total N gkg^{-1}	<	<	=	=
Av.P $mgkg^{-1}$	<	=	>	>
Exch.K $cmolkg^{-1}$	<	<	<	=
Total P $kgha^{-1}$	<	<	<	<
CEC $cmolkg^{-1}$	<	<	>	>
Bio-indicators				
Ew count m^{-3}	<	<	>	>

> increase; < decrease; = no change or stable; Vs. = versus; mth = month; yr = year; Bd = bulk density; Tp(%) = total porosity percent; PAWC (%v) = plant available water capacity on volumetric percent; C = carbon; N = nitrogen; Ext. P = extractable phosphorus; Exch. K = exchangeable potassium; CEC = cation exchange capacity; Ew = earthworm. Only dynamic soil properties were selected.

The LUTs of 10 and >22 years have higher values of total OC relative to the reference soil. This may be associated to management practices. Soil quality maintenance and improvement in the savanna soils of Nigeria would depend on sequestration of organic matter high in humic substances (Raji, 2011).

The values of soil properties presented in Table 4 is used to compare with critical limits reported by Ojanuga and Awojuola (1981), Akinrinde and Obigbesan (2000) and Ezeaku and Iwuanyanwu (2013).

Soil nitrogen as a soil quality is one of the key nutrients in plant production. Most important of all 16 essential plant elements needed for growth, development and reproduction and also the most easily limiting or deficient

in the tropics (Adaikwu et al., 2012). The values of total N in the 3 and 7 month LUTs are less than 0.15% or 1.5 g kg^{-1} total N at which response to N fertilization is not expected in the soils of the tropics. Low percentage soil N (0.15%) as obtained 3 and 7 month LUTs requires 200 kg ha^{-1} Urea in guinea savanna agroecology (Agbede, 2009) as cited by Adaikwu et al., (2012).

Available P is the second most critical element influencing plant growth and production. It is taken up by plants from soil solution as orthophosphate anion $H_2PO_4^-$ or HPO_4^-. In Table 4, the available P in 3-month and 7-month LUT is 4.2 and 2.9 mg kg^{-1}, implying low available P contents. For such LUTs in guinea savanna, 225 $kgha^{-1}$ of P is recommended for maize production if available P is low (<8.0 ppm) (Agbede, 2009) as cited by Adaikwu et al. (2012). However, across the plantation LUTs the values of P are greater than 8 to 12 mg kg^{-1} critical limit for the tropics, suggesting non-limiting P availability.

Exchangeable K values are higher than the critical values of 0.16 to 0.20 Cmol kg^{-1}. Cation exchange capacity (CEC) is a key component of any minimum data set to be used for assessing SQ and sustainability of agricultural management systems (Raji, 2011). This is because it determines the soil's capacity to hold and exchange natural and artificial sources of cationic plant nutrients. CEC is classified as low (< 6 Cmol kg^{-1}), medium (6-12 Cmol kg^{-1}) and high (> 12 Cmol kg^{-1}). Based on these limits, the amounts of CEC across all LUTs are generally low to medium (Table 4), suggesting high nutrient deficiencies that may be related to intense leaching and erosion due to rainfall, high mineralization rate and crop exports. The low levels signify response to N, P and K fertilization for the crops.

Contrast analysis results in Tables 5 and 6 shows that traditional lands use have modified the soil properties, especially in 3-month and 7-month LUTs. This indicates that change in soil quality indicators occurred and it is in synchrony with the observation that chemical, physical, and biological indicators of soil quality generally decline in response to intensive cultivation (Pandey, 1996). The soil characteristics most sensitive to 3-month and 7-month LUTs, showing significant differences at 0.05 probability level (t-test) with respect to the reference conditions were organic carbon, CEC, total porosity and PAWC. Even though these dynamic soil attributes could be used for biocycling, partitioning, storage and release of water and buffering of soil solution (Karlen et al., 1997), their amounts in the soil are generally low. This may be related to degradation resulting from land misuse and soil management. Soil degradation is the lowering of soil fertility to a threshold that cannot maximize agricultural productivity (Ezeaku et al., 2012).

On the other side of the spectrum, the observed soil properties of 10-year and >22-year LUTs were relatively unaffected because of probable suitable conditions. The result in Table 5 generally show that the contrasted 10-year and >22-year LUTs SQI were within the original reference levels, suggesting thus, it is unlikely that the soils are functioning much below their potentials, which indicate a high level of soil resilience and a greater ability of the soils to return to their original dynamic equilibrium after disturbance (Seybold et al., 1999).

Results indicate that bulk density, when contrasted to reference soil conditions, consistently decreased with relative increase in nutrient elements and volume of water in plantation LUT, an indicative improvement in SQ due to management. These phenomena may be associated to length of cultivation, increased microbial processes occasioned by added organic inputs and mulch management. These are expected to reduce compaction and favor infiltration over surface runoff, a probable justification for the decrease in bulk density (Tables 5 and 6) and a further suggestion that biological activity occurred, and storage and release of water can be altered. Increased microbial activities could be an induced change due to organic material management.

Lower bulk density (Bd) and earthworm number were deemed level 1 indicators of a soil's ability to accommodate water entry for prolonged periods during high-intensity rainfall and frequent irrigation events (Karlen et al., 1997). Furthermore, number of earthworms can indicate the extent of macropores (earthworm burrows) able to quickly drain surface water hence low bulk density indicates a high volume of water. Based on baseline and threshold values for earthworms (determined from population counts in the 10-year and >22-year orchards and related to the fallow), Table 6 show that the level of microbial activity and thus nutrient cycling may increase more in >22-year LUT relative to the 10-year land use because of the number of earthworms observed. However, the total number of earthworms is below the baseline of 50 or 100 for integrated plots (Werner, 1996).

Conversely, the increase in nutrient elements and especially total-P in plantation LUTs is a result of long-term supply of organic matter applications and represents a management-induced enhancement of soil quality. The increases may be beneficial as phosphorus plays critical role in plantation growth and fruit productivity, although at high levels, however it may interfere with uptake of Ca by trees, and to be more susceptible to loss of surface water runoff (Sharpley, 1996).

Recovery of SOM after the cessation of human activity would seem to be favored more in plantation LUTs than cereal and arable LUTs because of probable higher average OC contents, less erosion and cold (pseudo-temperate) climate condition occasioned by mulch and plant canopy formed. Presence of soil organic matter may impact on physical and chemical properties of plantation soils and thus may likely play a crucial role in their resiliency. Again, the high sequestered organic carbon in the plantation LUTs raises potentials for SQ improvement, which consequently may serve to mitigate the effect of climate change. This can be catalogued as

Table 7. Diagnostics of soil quality indicators (SQI) based on farmers experiences.

Soil indicators	Qualitative SQI used by farmers
SOM	Dark color and good aromatic smell.
Fertility	Based on yield and plant growth (Biomass). Lush green leaves indicate high fertility, stunted growth suggests poor fertility.
Compaction	Hard and dry when touched or feeled.
Structure	Observed soil crumbs during cultivation is a good structure.
Consistence	Stickiness on hoes when cultivating.
Moisture	Observed moist feels and dews on leaves at morning periods
Surface soil thickness	Observing the depth of dark colored soil during hoeing.
Soil erosion	Observing soil surface after rainfall event; comparing yearly variations in topsoil depth during ploughing.
Weed incidence	Presence of weed species in the field.
Earthworm population	Observing number of earthworm casts at the soil surface.

sustainable land use.

Qualitative soil quality indicators

Most of the farming operations (e.g., weeding, fertilizer application and harvesting) involved in the different land uses was carried out manually. There were no reports of serious labour shortages in the crop production areas but could be costly during peak demands. In any case, the farmers had performed farm works by themselves. As working in their crop production fields for a long-time farmers know their soil indicator best. The criteria farmers used to assess changes in soil quality are described in Table 7. Farmers commonly assess soil quality in terms of visual properties of the soil, such as appearance, feel or taste. For example, observed changes in soil color (darkness) are used by farmers to evaluate changes in organic matter content. Likewise, soil water content is assessed by feeling the soil. Plant growth and crop yield were used for fertility criteria. Many farmers perceived that their soils were still fertile if crop yields were comparable to those achieved in previous years with the same management level.

Farmers considered that drop in productivity following long-term cultivation could be attributed to degradation of the soil quality. This is because the yield potential of the crop plants remained good even after years of cultivation, provided an adequate supply of plant available nutrients was maintained through adequate fertilization to the crop land uses. The occurrence of some wild plant species in the crop fields was a useful indicator of some soil properties. Experienced farmers linked the presence of certain weed species (e.g., *Mimosa pudica* and *Eupatorium odoratum* L.) to increased acidity. Likewise, species such as Spear grass; C*hrysopogon aciculatus* R. etc. were used as indicators of poor nutrient status (soil fertility) and dryness of the soil, both of which are indicators of soil degradation. However, the use of wild plant indicator to judge soil acidity may have some limitation whereas occurrence of some species (e.g. *E. odoratum* L) may be due to not only soil acidity, but also the changes of other soil properties (that is, soil moisture and soil fertility) and/or the changes of crop canopy with time (Ezeaku and Salau, 2005).

Farmers were asked to comment on ten indicators of soil quality (Table 7). Most recognized that organic matter content, soil fertility, soil moisture storage, soil structure, earthworm population, and weed incidence decreased over time, while soil compaction increased as a result of long-term cultivation. It is apparent that these soil indicators were well recognized and easily assessed by farmers, hence could serve as soil health card (Romig et al., 1995; Mausbach et al., 1998).

In contrast, changes of other soil indicators such as thickness of topsoil, and soil erosion were not well recognized by many farmers and their answers varied from farmer to farmer (e.g. 29% of interviewed farmers indicated that soil erosion increased along with time of cultivation, while 58% considered soil erosion decreased) (Table 8). The response of many farmers about changes of these soil quality indicators do not agree with scientific approach such as the use of USLE or EPIC models. This is possibly because these soil indicators were not easily recognized by observation, and the criteria used to assess changes of these soil quality indicators were too complicated and unsuitable with farmers' knowledge. This may be a limitation of farmer approach to evaluate soil quality.

Each farmer was asked to rank generally the relative importance of the various soil quality indicators as it relates to their crop production. They ranked the SQI in the following increasing order of importance (Table 9): Soil organic matter content, soil fertility, topsoil thickness, structure, moisture, earthworm, compaction, soil erosion, and weed incidence. The last three SQ indicators are

Table 8. Farmer perceptions of change in soil properties with crop cultivation (expressed as a percentage of 22 farmers).

Indicator	Increase	Decrease	No change	No idea
SOM	30	57	13	0
Fertility	26	54	18	2
Compaction	42	14	31	3
Structure	14	66	20	0
Consistence	24	53	17	6
Moisture	38	44	18	0
Surface soil thickness	22	61	17	0
Soil erosion	29	58	6	7
Weed incidence	26	57	14	3
Earthworm population	4	9	86	1

SOM = soil organic matter; pop. = population.

Table 9. Ranking of soil quality indicators based on farmers' perceptions.

Indicator	Total SQI points**	Overall rank
SOM	90	1
Fertility	104	2
Topsoil thickness	136	3
Structure	178	4
Moisture	193	5
Earthworm	208	6
Compaction	235	7
Soil erosion	271	8
Weed incidence	294	9

Each farmer ranked the SQI on a scale from 1 to 9, with 1 being the most important indicator, and 9 being the least important. Soil quality points for each indicator were then totaled, and an overall ranking assigned to each soil variable.

considered the least important but are important in conservation programs for soil protection and productivity enhancement.

Conclusion

The study revealed that depletion of the soil nutrients, particularly N, P and K, due to continued cultivation with imbalanced fertilization caused a degradation of SQ in short-term (3-month and 7- month) land uses. Opposite was the case for 10-year and 22-year plantation LUTs. The statistical mean values of the bio-physical and chemical properties of SQIs under the various LUTs show that soil pH, total organic C, available phosphorus (P), CEC, bulk density, total porosity, and PWAC, and earthworm population were the most sensitive soil quality indicators ($P \leq 0.001$). Moderate sensitive indicators were total N, P and K. Percentage BS showed weaker indicators of SQ, while soil texture and clay/silt ratio were of no value as soil quality indicators for these soils. In terms of SQI improvements with applied management practices, LUT4 had the best SQ followed by LUT3, while LUT1 had the worst management. Qualitative assessments based on farmers' perception of SQ showed that farmers considered organic matter, inherent fertility, topsoil thickness, structure, PAWC and biochemical processes (earthworm activities) as important soil quality indicators for increased crop production. Consequently, soil conservation programs targeted at crop growers should address all the factors identified. Evaluating SQ using bio-indicator (earthworm count as biological factor) underlines the importance of process-related microbial and physicochemical parameters in evaluating ecological SQ indicators. The methodological approach presented and discussed in this study should further strengthen national, regional and an international attempt in harmonizing of procedures for the monitoring and evaluation of soil quality. Farmer observations of SQ changes were generally in good

agreement with the quantitative assessments. To ensure adoption of improved management practices for more sustainable production system, qualitative soil quality information obtained from on-farm surveys should be used to supplement the quantitative data obtained through soil analyses. Both assessment methods provided important information that could be used as entry point for wider geospatial application. Using both assessment methods could also serve as effective diagnostic tools for evaluating soil quality for long-term sustainability of crop productivity. This would equally allow the development of soil quality standards and control techniques, and subsequently the design of sustainable land management systems.

Conflict of Interest

The authors have not declared any conflict of interest.

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